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Genetic Impact on First Line Treatment to Hypertension in Black South Africans

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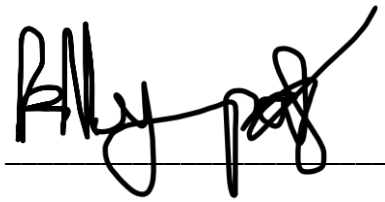
Professor Collen Masimirembwa, Dr Andrew May and Dr David Twesigomwe

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, in fulfilment of the requirements of the degree of Doctor of Philosophy in Human Genetics

Johannesburg, 2025

Declaration

I, Kudakwashe Nyamupangedengu, declare that this thesis is my own work. Where others have contributed and assisted, they have been duly acknowledged. This thesis is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at this, or any other university.



27/09/2025

This thesis is dedicated to:

Mbuya Mai J and Simbarashe Chekwa.

The bravest woman and the bravest girl I have ever known,

Through me you've lived on,

But now it is forever.

Presentations arising from this research project

Nyamupangedengu K, Twesigomwe D, May AK, Martinson N, Waja Z, Masimirembwa C, Ramsay M. Genetics impact response to hypertension treatment in Black South Africans. Poster presentation at the ICHG Conferences, January 2023, Cape Town, South Africa

Nyamupangedengu K, Twesigomwe D, May AK, Martinson N, Waja Z, Masimirembwa C, Ramsay M. Genetics impact response to hypertension treatment in Black South Africans. Poster presentation at the Sydney Brenner Symposium, 9 April 2024, Johannesburg, South Africa

Nyamupangedengu K, Twesigomwe D, May AK, Martinson N, Waja Z, Masimirembwa C, Ramsay M. Genetics impact response to hypertension treatment in Black South Africans. Oral presentation at the Johannesburg Health District PHC Research Conference, 28-29 August 2024, Johannesburg, South Africa

Nyamupangedengu K, Twesigomwe D, May AK, Martinson N, Waja Z, Masimirembwa C, Ramsay M. Genetics impact response to hypertension treatment in Black South Africans. Oral presentation at the Wits University Faculty of Health Research Day, 5 September 2024, Johannesburg, South Africa

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Nyamupangedengu K, Twesigomwe D, May AK, Martinson N, Waja Z, Masimirembwa C, Ramsay M. Genetics impact response to hypertension treatment in Black South Africans. Oral presentation at the 3 Minute Thesis Competition, 30 October 2024, Johannesburg, South Africa

Nyamupangedengu K, Twesigomwe D, May AK, Martinson N, Waja Z, Masimirembwa C, Ramsay M. Genetics impact response to hypertension treatment in Black South Africans. Oral presentation at the Wits University MBRT Research Day, 7 December 2024, Johannesburg, South Africa

Publications arising from this project

The research and findings arising from this study were drafted into two manuscripts. Both have been accepted for publication in international journals. Nyamupangedengu K, Twesigomwe D, May AK, Martinson N, Waja Z, Osman R, Masimirembwa C & Ramsay M (2024) Hypertension treatment practices and outcomes at health community centres in urban Soweto, South Africa. ***Cardiovascular Journal of Africa***

Nyamupangedengu K, Twesigomwe D, May AK, Martinson N, Waja Z, Letutu M, Masimirembwa C & Ramsay M (2025) Genetic Variation in Hydrochlorothiazide Response-Related Genes among Hypertensive Individuals in Soweto, South Africa. *Clinical and Translational Science.* Volume 18, Issue 7, https://doi.org/10.1111/cts.70302

Awards

2024: Recipient of Johannesburg Health District PHC Research Day best research award

2024: Recipient of 3rd place 3 Minute Thesis Competition, Faculty of Health Science, University of the Witwatersrand (R5 000)

2024: Recipient of 3rd place oral, MBRT Research Day, University of the Witwatersrand (R1 000)

Abstract

Background: Systemic arterial hypertension (HT), a condition characterized by systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic BP (DBP) ≥ 90 mmHg, is the leading modifiable risk factor for cardiovascular diseases (CVD). Africa carries the largest burden of HT worldwide, with recent studies reporting prevalence as high as 48%**.** There is growing evidence that genetic variation can affect interindividual response to HT medications, including diuretics and calcium channel blockers which are commonly prescribed for African-ancestry patients. This adds to the HT treatment burden in Africa. This thesis therefore investigates (1) HT treatment practises in South African health primary health centres and (2) the potential role of pharmacogenetic variation in treatment response in Black South African patients.

Methods: An observational prospective study on newly diagnosed hypertensives undergoing treatment at clinics in Soweto, South Africa, was conducted. Anthropometric, demographic, and clinical data were recorded at baseline and during subsequent follow up visits. Fisher's exact and Kruskal-Wallis tests were conducted to test for associations between clinical variables and treatment subgroups. Linear mixed models for longitudinal data were used to identify predictors of blood pressure at first follow up. For the genetic analyses, targeted next generation sequencing was conducted on 16 candidate genes, selected due to their importance in the response to the first-line diuretic drug hydrochlorothiazide (HCTZ). Following variant calling, in silico tools were used to identify potentially deleterious variants. Furthermore, association tests were performed to determine potential variant signals for the response (or non-response) to treatment.

Results: A total of 364 participants were included in the study having met the main criteria of at least one follow-up visit. There were more female participants compared to males and the mean age was 49 years. Combination therapy was prescribed more frequently than monotherapy, and thiazide diuretics and calcium channel blockers were the most prescribed drug classes. There was a significant decrease in the mean systolic and diastolic blood pressure between baseline and first follow up across all treatment subgroups. More than half of the study participants had controlled blood pressure at first follow up. There were clinic-specific differences in treatment strategy (regarding number of drugs and drug class prescribed) and this was reflected in the outcomes. More females had controlled blood pressure at first follow up compared to males. For the genetic analyses, a total of 1 258 variants were identified across the cohort, with 15% (188/1258) in coding regions and 7% (89/1258) being novel. Of the novel variants in coding regions, 66% (8/12) were predicted to be deleterious by at least one computational variant effect predictor. Lastly, of the eight variants previously reported to influence HCTZ response (in European, American and Asian populations), none were associated with HT treatment outcome in our study. Moreover, two of these variants (rs10995311 and rs5186) were rare in our cohort but common in the European and Asian cohorts.

Discussion: This study is the first to explore the characteristics of newly diagnosed hypertensives and treatment practises at primary health care level in Soweto, South Africa. The disease landscape of HT in a community like Soweto is complex and the management

practices adopted reflect this. The genetic variation identified in HCTZ response-related genes in this cohort exemplify the rich genetic diversity in continental Africans. While computational tools provided insights on the effects of variants across the candidate genes, the clinical functional impact, especially for predicted deleterious variants, needs to be further assessed to better understand their potential influence on response to treatment.

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List of abbreviations

ACE – angiotensin-converting enzyme

ACEi – ACE inhibitors

ADME – Absorption-Distribution-Metabolism-Excretion

AGTR1 – Angiotensin II receptor type I

AIC – Akaike Information Criterion

ARB - Angiotensin II receptor blockers

BIC – Bayesian Information Criterion

BMI – body mass index

BP – Blood pressure

CCB – Calcium channel blockers

CVD – Cardiovascular disease

DNA – Deoxyribose nucleic acid

ENaC - epithelial Na+ channels

GATK – Genome Analysis Toolkit

GVCF – Genome variant call file

GWAS – Genome-wide association studies

HCTZ – Hydrochlorothiazide

HIV/AIDS – Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome

HT – Hypertension

MEM – Maximal exact matches

NCC - NaCl co-transporter

NGS – Next generation sequencing

NKCC2 - NA+-K+-2Cl- cotransporter 2

PTH – Parathyroid hormone precursor

RAAS – Renin-Angiotensin-Aldosterone System

SNP – Single nucleotide polymorphism

SNS – Sympathetic nervous system

VEP – Variant effect predictor

WHO – World Health Organization

Preface

This PhD thesis is presented in a divided block format with two manuscripts. One manuscript has been accepted for publishing in an internationally peer-reviewed journal and the other manuscript has been submitted for publication.

The thesis is divided into four chapters:

1. Introduction – this chapter explores the literature on hypertension, pharmacogenomics and genomics of hypertension treatment response. Soweto, South Africa, the lens through which the work was performed, is also described herein. This chapter concludes with the study aims and objectives and methodological approaches used.
2. Hypertension treatment practices and outcomes at health community centres in urban Soweto, South Africa – this is the first publication and first results chapter. Here, we described the hypertension treatment practices and treatment outcomes we observed in our study.
3. Genetic Variation in Hydrochlorothiazide-Response Related Genes among Hypertensive Individuals in Soweto, South Africa – this is the second manuscript and second results chapter. Here, we detailed the results of our analysis on the impact of genetics on treatment response and outcomes in black South African hypertensives.
4. Discussion and Conclusions – this chapter contains a summary of the key findings of the study, research themes emerging from the study, limitations and potential future studies. This chapter ends with an overall conclusion of the achievements of the study.

Chapter 1: Introduction

Hypertension (HT) is a global health concern, increasing the risk for cardiovascular and metabolic diseases. The prevalence of HT is on the rise, with Africa disproportionally affected, providing the impetus for this study. Studies continue to show that individuals of African descent respond differently to certain antihypertension drug classes compared to other populations. The purpose of the study was to understand treatment approaches and outcomes for HT in urban health clinics in Soweto (South Africa) and to identify genetic variants that may affect response to treatment.

1.1 Hypertension overview

Hypertension is a condition characterized by persistently high blood pressure (BP). Blood pressure is measured as two values: systolic and diastolic BP (pressure in the arterial wall when the heart contracts and relaxes, respectively) (Oparil et al., 2018). According to World Health Organization (WHO) estimates, 1.3 billion people were affected by HT globally in 2023, with 78% of them living in low- and middle-income countries (WHO, 2023). Despite the high burden of HT, Africa is the region with lowest percentage of hypertensives undergoing treatment (27%) and with the lowest levels of controlled blood pressure (12%) (Table 1).

Table 1. 1: Prevalence, diagnosis, treatment and control amongst adults between 30 and 79 years of age, across different regions (WHO, 2023)

Region	Prevalence (%)	Diagnosed (%)	Treatment (%)	Controlled[1] (%)
Africa[2]	36 (33,38)	43 (39,43)	27 (24,30)	12 (9,14)
The Americas[2]	35 (33,38)	70 (67,73)	60 (57,64)	36 (32,41)
South-east Asia[2]	32 (29,36)	39 (34,44)	30 (25,34)	14 (10,18)
Europe[2]	37 (35,39)	66 (63,69)	53 (50,56)	26 (23,29)
Eastern Mediterranean[2]	38 (35,41)	49 (45,53)	39 (34,43)	15 (13,19)
Western Pacific[2]	28 (25,32)	54 (48,59)	41 (35,47)	18 (14,23)
Global	33 (32,35)	54 (51,56)	42 (40,45)	21 (19,23)

[1] Controlled hypertension defined as blood pressure below 140mmHg (systolic) and below 90mmHg (diastolic)

[2] n (95% CI)

The most prevalent form of HT is primary HT (also referred to as essential HT), with 90-95% of patients with high blood pressure falling within this category (Oparil et al., 2018). The cause of essential HT is often unknown, and its aetiology is multifactorial involving genetic, behavioural, and environmental factors. Secondary HT describes the condition where a patient's blood pressure increases because of an identifiable cause (Carretero and Oparil, 2000). Secondary HT is often easier to control once the cause is known. Common causes of secondary HT are hormonal or arterial abnormalities, disease, alcohol or salt intake, and certain drugs. The other forms of HT within these two categories are isolated systolic, malignant, and resistant HT. Resistant HT is characterized by non-response to any antihypertensive medication and is often genetically driven or caused by an underlying disease. Malignant HT occurs as a rapid rise in HT and is often a medical emergency (Naranjo et al., 2022). Isolated systolic HT, prevalent in individuals over 65, is caused by loss of elasticity in arterial walls and is characterized by a systolic BP over 140 mmHg and a normal diastolic BP below 90 mmHg (Staessen et al., 2000). Essential HT is the result of the body's natural mechanisms of homeostasis failing to compensate for all the factors which increase blood pressure.

HT is one of the predominant risk factors for developing chronic kidney disease, aortic aneurysm, atrial fibrillation, congestive heart failure and other cardiovascular diseases (CVDs) (Tackling and Borhade, 2024). For every 20 mmHg systolic and 10 mmHg diastolic blood pressure over the threshold of 120/80 mmHg, the risk of cardiovascular mortality doubles in middle and old age (Lewington et al., 2002). Atherosclerosis (hardening of the arteries caused by a build-up of plaque in the lining of an artery) is key feature in CVDs and is characterized by increasing arterial thickness (Lusis, 2000). Increased arterial thickness is higher in hypertensives compared to normotensives (Magnussen, 2017), emphasizing the importance of HT in CVD risk and mortality. Furthermore, HT has been reported to be associated with insulin resistance, oxidative stress, dyslipidaemia, and hyperuricemia which all increase the risk of CVDs (Cooper et al., 2007; Tasic and Lovic, 2018; Sadikov et al., 2023).

1.2 Aetiology of hypertension

HT involves combinations of modifiable and non-modifiable factors which are connected in a complex way. Genetics, age, ethnicity, sex and early foetal development are non-modifiable factors which influence the risk of developing HT. The relative risk associated with these non-modifiable factors is exacerbated by modifiable environmental and lifestyle factors such as dietary salt, stress, sedentary behaviour and obesity. When combined, these factors result in elevated BP through pathophysiological mechanisms such as renal and endothelial dysfunction and sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS) dysregulation. This complex multifactorial interplay makes HT difficult to diagnose, treat and manage.

1.2.1 Homeostasis control of HT

This sub-section will explore the normal physiological mechanisms for controlling blood pressure and maintaining homeostasis control. These include the renin angiotensin aldosterone system, the sympathetic nervous system and renal system.

1.2.1.1 Renin Angiotensin Aldosterone System

The Renin-Angiotensin-Aldosterone system (RAAS) is the primary blood pressure regulator, controlling blood volume, sodium and water reabsorption, potassium secretion, and vascular tone (Fountain and Lappin, 2022). RAAS is initiated by the conversion of prorenin to renin in the juxtaglomerular cells of the kidney in response to a decrease in blood pressure (Reid, 1984). Once renin enters the bloodstream it activates a cascade of protein-conversions and signalling pathways which result in angiotensin II, the active form of angiotensinogen (Bernstein et al., 2018). Angiotensin II elicits blood pressure-increasing effects by acting on the kidney, adrenal gland, arterioles, and brain. In the kidneys, angiotensin II increases Na-H exchange in the convoluted tubule, thus increasing the osmolarity in the blood, leading to fluid shifting from the tubule into the blood (Harrison-Bernard, 2009).

Aldosterone is a steroid hormone which is released after stimulation of the adrenal cortex by angiotensin II. Aldosterone affects sodium reabsorption and potassium excretion by facilitating the insertion of sodium channels and NA-K ATPase proteins into the cell membrane (Harrison-Bernard, 2009). Angiotensin II causes vasoconstriction in the systemic arterioles through binding to G-protein coupled receptors and triggering secondary messenger signalling pathways (Fountain and Lappin, 2022). Angiotensin II also acts in the brain by binding to the hypothalamus to stimulate thirst and water intake. The actions of angiotensin II on the brain also include stimulating the release of antidiuretic hormone to increase water reabsorption and decreasing baroreceptor sensitivity. Baroreceptor acts to inhibit the effects of angiotensin II (Reid, 1984).

Interestingly, there is evidence that activation of the RAAS works in tandem with SNS activation to increase blood pressure. In the kidneys, increased renal sympathetic nerve activity increases renin production which results in increased RAAS activity (Cabandugama et al., 2017). Activated RAAS activity in-turn inhibits norepinephrine reuptake at the sympathetic nerve terminals, thus maintaining the activated SNS state (Raasch et al., 2001).

1.2.1.2 Sympathetic Nervous System

Increased sympathetic nervous system (SNS) activity is an important mechanism driving HT. SNS activation influences can be categorized as cardiac, vascular, adrenal and renal (Lambert et al., 2019). *Obesity-associated SNS activity predominantly affects skeletal muscle and kidneys* (Hall et al., 2010). Norepinephrine is the main neurotransmitter of the SNS, with epinephrine and other transmitters performing supporting functions (Charkoudian and Rabbitts, 2009). Cardiac SNS activation occurs through activation of the sinoatrial node, allowing the SNS to alter the diastolic depolarization slope during the node action potential resulting in an increase in heart rate (Charkoudian and Rabbitts, 2009). SNS activation can also impact the myocardium, increasing contractility and stroke volume as a result (Charkoudian and Rabbitts, 2009). The activity of norepinephrine at postsynaptic α-adrenergic receptors of vascular tissue, such as veins and arteries, causes vasoconstriction and increased blood pressure (Stephens et al., 2001). SNS activation primarily triggers the adrenal medulla to secrete norepinephrine and epinephrine into the body, increasing blood pressure through several mechanisms (Joyner et al., 2008). Renal sympathetic nerve activity increases sodium reabsorption and water reuptake maintaining high blood volume and blood

pressure (Hall et al., 2010). SNS activation regulation is crucial in the maintenance of blood pressure homeostasis and is often central in the mechanism of action of many risk factors of increased HT, such as obesity.

1.2.2 Risk factors of HT

This sub-section will explore the risk factors for developing HT, how they interact and relate to each other as well as how their effects disturb the normal homeostasis responsibilities of HT regulatory pathways.

1.2.2.1 Obesity

Obesity is one of the main risk factors for increased blood pressure. The nature of obesity is a nuanced and ever-evolving biological concept, complicating our understanding of its relationship with HT. Obesity is often measured by body mass index (BMI), which is an individual's weight (in kilograms) divided by their height (in metres^2), a definition that is known to be flawed but widely used because of its ease of measurement. Although BMI is the standard tool for diagnosing obesity, it does not provide information about body fat and its distribution, nor does it shed light on the health or illness for the individual (Rubino et al., 2025). BMI in conjunction with body size measurements (i.e. waist circumference or waist-to-hip ratio) and assessments of organ function and ability to do standard everyday activities may lead to more accurate and clinically relevant diagnoses of obesity (Rubino et al., 2025).

Obesity is caused by a combination of genetics, diet, lack of exercise, and psychological factors, such as depression, which results in an imbalance in energy intake and expenditure (Rosenbaum and Leibel, 1998). Obesity-related HT is well described across various ages and populations. In the American population, excess body fat (as a measure of obesity) was attributed as the main contributing factor to 78% of new HT cases in men and 65% in women (Garrison et al., 1987). In the same population, HT prevalence increased with weight irrespective of race and ethnicity (Young et al., 2018). In the Latin-American population, longitudinal studies have found obesity prevalence to be higher in Brazil (35%), Mexico (60%), Paraguay (68%), Peru (53%) and Argentina (35%) (Filozof et al., 2001) and a positive association between obesity prevalence and HT has been previously reported in these countries (Ferreira et al., 1997; Gigante et al., 1997; Trindade et al., 1998). In the European population, 59% of adults are living with obesity with males carrying more of the burden compared to women and this sex-related pattern is also seen in the prevalence of HT in the population, both in magnitude (55%) and sex-specific burden (Beaney et al., 2018). Although the prevalence of HT directly related to obesity is unknown, the increase in prevalence of obesity has been correlated with the increase in prevalence of HT (Beaney et al., 2018). There is a clear epidemiological relationship between obesity and HT such that the prevalence of one informs the prevalence of the other. This therefore suggests that understanding obesity is crucial to understanding HT.

Obesity affects SNS activation. Angiotensin is secreted in adipose tissue (Engeli et al., 2003) and therefore high levels of adipose tissue lead to increased angiotensin secretion. Adipose tissue excretes all components of the RAAS system including renin and angiotensin-converting enzyme (ACE) and therefore could lead to increased blood pressure. Several studies have shown increased sympathetic nerve activity in patients with obstructive sleep apnea

(Narkiewicz and Somers, 2003), a condition commonly associated with obesity. Obese individuals have narrowed respiratory tracts due to fat deposits and therefore are highly susceptible to sleep apnea (Jehan et al., 2017). Leptin is a peptide hormone cytokine, made by adipocytes, which regulates appetite and energy consumption through SNS activation. Obese individuals have been shown to have high levels of leptin but resistance to its appetite suppression and metabolic effects (Considine et al., 1996). Leptin resistance is another side effect of obesity which stimulates SNS activity and increases BP.

There is an illustrated synergy between obesity and HT, and SNS activation is integral to increased blood pressure. This is supported by findings that α- and β-adrenergic blockers reduce BP significantly more in obese hypertensives compared to lean hypertensives (Wofford and Hall, 2004). Obese individuals often suffer from obstructive sleep apnea, leptin resistance and even insulin resistance which all act to drive the positive feedback loop between SNS, RAAS activation and increased blood pressure.

1.2.2.2 Insulin resistance

Insulin resistance is a condition where the body does not respond normally to insulin. Insulin is a hormone excreted by the pancreas and it is responsible for moving glucose from the blood into cells for energy or storage and for inhibiting liver glucose production (Freeman et al., 2025). Insulin resistance results in glucose accumulation in the blood and the pancreas adjusts by producing more insulin (Freeman et al., 2025). This is called hyperinsulinemia. Excess body fat is the primary cause of insulin resistance with other secondary causes including genetics, physical inactivity, diet and certain medications (Harano et al., 2002).

The increased adipose tissue in obese individuals often triggers pathophysiological mechanisms for cardiometabolic disorders such as insulin resistance. Adipokines such as leptin and adiponectin are hormones produced by adipocytes (Mullen et al., 2007). Adiponectin increases AMP-activated protein kinase activity, enhancing uptake and oxidation of fatty acids and glucose, leading to expending of energy and reduced accumulation of fat (Mullen et al., 2007). Leptin is best known as an appetite suppressor but also plays a role in fat oxidation by activating AMP-kinase and other metabolic pathways (Mullen et al., 2007). Fat oxidation facilitated by adipokines reduces inflammation and activates pathways which in-turn improve insulin receptor activity, as well as supports better glucose homeostasis by making fatty acids an available energy source (Tong et al., 2022). Adipokines therefore play a crucial role in insulin sensitivity. However, increased production of adipokines, due to excess adipocytes, in obese individuals often leads to adipokine resistance (Enriori et al., 2006) which is characterized by poor insulin receptor activity, and reduced fat oxidation. Ultimately, this results in insulin resistance.

Similarly, the excess adipose tissue in obese individuals produces pro-inflammatory molecules such as tumour necrosis factor-alpha (Hotamisligil et al., 1993) and interleukin-6 (Fried et al., 1998). This obesity-induced pro-inflammatory state leads to insulin resistance either directly by defecting tyrosine kinase activity and inhibiting insulin signalling (Ros Pérez and Medina-Gómez, 2011) or indirectly through secretion of glucagon-like peptide in intestinal and pancreatic cells (Ellingsgaard et al., 2011). Additionally, the insulin pathways that control the ability of capillary blood networks to sufficiently take up glucose, can be disrupted by

adipokine overproduction (like in the case of obese individuals) leading to insulin imbalance and cause insulin resistance (Muris et al., 2013).

This relationship between obesity and insulin resistance is linked with HT. Literature suggests that insulin resistance can act on blood pressure homeostasis. Insulin resistance and compensatory hyperinsulinemia increase sodium reabsorption in the kidneys (DeFronzo, 1981). This leads to an increase in circulating blood volume, which can contribute to increased blood pressure. Elevated insulin levels in response to insulin resistance stimulate the sympathetic nervous system leading to increased heart rate, vasoconstriction and high blood pressure (Esler et al., 2001). Elevated insulin levels also directly affect endothelial function and the production of nitric oxide, a vasodilator (Wu and Meininger, 2009). Reduced nitric oxide availability would therefore contribute to high blood pressure through reduced vasodilation. Elevated insulin levels can also increase the stimulation of the RAAS pathway, with RAAS proteins such as angiotensin II causing vasoconstriction and sodium retention, both of which raise blood pressure (Zhou et al., 2012).

The relationship between obesity and insulin resistance and their link to HT is of great clinical importance. Insulin resistance is a key feature of metabolic syndrome; a group of conditions which raise the risk of cardiovascular disease, stroke and other serious health problems (Bowo-Ngandji et al., 2023). Metabolic syndrome makes the management of HT more challenging. On the African continent, metabolic syndrome is most prevalent in women, urban populations, and populations in Southern Africa (Jaspers Faijer-Westerink et al., 2020; Bowo-Ngandji et al., 2023). Insulin resistance and HT independently increase the risk of cardiovascular diseases and when combined with other conditions in metabolic syndrome, the risk of cardiac arrests, stroke and other cardiovascular events increases exponentially. Addressing insulin resistance through diet, exercise and weight loss is crucial for effectively managing HT and reducing the risk of cardiovascular disease. Furthermore, HT medications such as ACE inhibitors (Çelebi Bitkin et al., 2013) and angiotensin II receptor blockers (Perlstein et al., 2012) also improve insulin sensitivity and reduce the risk of cardiovascular events from metabolic syndrome.

1.2.2.3 Age

HT (and obesity) also has common risk factors such as increasing age, sedentary lifestyle, high-salt diet, alcohol intake and genetics. Obesity increases with age, with worldwide survey data indicating that adult obesity prevalence is 21% higher than adolescent obesity and 19% higher than the prevalence in children aged 5-9 (Elflein, 2020). In a meta-analysis of studies conducted in 114 African countries, with a mean age range of 65-79, the prevalence of obesity was as high as 50% in South Africa (Mabiama et al., 2022). Similarly, the risk of HT (Kumar, 2013), treated but uncontrolled HT (Borzecki et al., 2006) and HT-linked cardiovascular disease (Fuchs and Whelton, 2020) increases with increasing age. The most prevalent type of HT in adults older than 50 is isolated systemic hypertension (Pinto, 2007). This is largely attributed to age-related arterial stiffness and narrowing (Singh et al., 2025). Endothelial dysfunction and SNS/RAAS overactivity also increase with age (Herrera et al., 2010; Balasubramanian et al., 2018), and therefore contribute to age-linked HT. Several studies

have confirmed increased HT risk with increasing age (Burt and Harris, 1994; Stamler and Neaton, 2008; Mahmood et al., 2014) emphasizing the clinical importance of this relationship.

1.2.2.4 Modifiable factors

Diet and lifestyle factors, such as smoking, drinking and exercise play a considerable role in the risk of developing HT. This role is mainly indirectly through obesity, and obesity-related effects. Sedentary behaviours such as excessive time spent watching television, computer gaming and physical inactivity were reportedly prevalent in obese individuals and also increased the risk of obesity in a dose-dependent manner (Hu et al., 2003; Heinonen et al., 2013; Silveira et al., 2022). Relatedly, sedentary behaviours were also associated with HT in South America (Tebar et al., 2022), North America (Spehar et al., 2020), Europe (Beunza et al., 2007), Asia (Gamage and Seneviratne, 2021) and Africa (Twinamasiko et al., 2018). There is no evidence of physiological mechanisms linking sedentary behaviours and HT but the shared association with obesity, suggests a possible relationship.

Similarly but inversely, a high sodium diet has previously been associated with obesity (Ma et al., 2015; Lee et al., 2023) although the exact causal mechanism is unknown. However, the mechanisms with which high salt intake influences increased blood pressure are better understood. High salt intake increases thirst (Stachenfeld et al., 1996, 1997) as an evolutionary response to maintain fluid homeostasis. This often leads to increased fluid intake, which increases blood volume. Similarly, high salt intake also leads to increased sodium retention in the kidneys (Rakova et al., 2017), which in turn results in fluid retention and increased blood volume. An increase in blood volume elevates blood pressure as the blood vessels of the circulatory system expand in response to this increase. High blood pressure over an extended period of time can cause arterial stiffness (Kim, 2023), as the blood vessels will lose the ability to contract and expand in response to changes in blood pressure. Interestingly, this relationship is bidirectional, as arterial stiffness also leads to elevated blood pressure.

The effects of salt on blood pressure are not homogenous. Salt-sensitivity is a physiological characteristic where blood pressure increases significantly with increasing salt intake (Elijovich et al., 2016). The biological mechanisms of salt sensitivity are complex and not well known. Salt sensitivity is strongly predicted by sex, age, and race (GenSalt Collaborative Research Group, 2007). Blood pressure in females has been previously showed to respond more to dietary sodium interventions compared to men (He et al., 2009). Salt sensitivity also increases with increasing age (Luft et al., 1991). Salt sensitivity is also more prevalent and severe in non-European individuals (Richardson et al., 2013). The genetic contribution to salt sensitivity in individuals with African ancestry is a topic of great interest and one that continues to be intently investigated. Genetic factors have been suggested to contribute as much as 74% to salt sensitivity in Black American individuals (Svetkey et al., 1996). Parathyroid hormone precursor (*PTH*) and Angiotensin II Receptor, Type I (*AGTR1*) were ranked top amongst salt-sensitivity genes with significant variability between continental Africans and European individuals (Tiffin et al., 2010). Haplotype and allele frequencies of genetic variants in *GRK4* (which encodes serine-threonine kinase, responsible for desensitization of the G protein (Jose et al., 2002), were observed to be different in Asians and African American

populations compared to Hispanics and Europeans, adding evidence to the gene's role in the predisposition of salt sensitivity (Williams et al., 2004). Epigenetic factors have also been suggested to contribute to salt sensitivity, although a link to particular populations has not been identified (Williams et al., 2012). It is clear that salt sensitivity is a consequence of gene-diet interactions. However, much work still needs to be done to find the genes and variants responsible for salt sensitivity in high-risk groups such as postmenopausal women, older individuals and individuals of non-European ancestry. Salt-sensitivity is also an excellent example of how complex the interaction network between genetics, diet and ethnicity can get when related to multi-factorial conditions such as HT.

1.2.2.5 Ethnicity

Ethnic differences in the prevalence of cardiovascular diseases including HT have been previously identified (Martin et al., 2015). In Africa, one study estimated the overall prevalence of HT as 57% in 2018 with the highest prevalence in the southern African sub-region (Bosu et al., 2019). HT prevalence was higher with increased age and BMI (Bosu et al., 2019). Data, from the Cardiovascular H3Africa Innovation Resource, showed that obesity was associated with 2-fold increase in HT in both sexes, on the continent (Akpa et al., 2020). In this study and others, obesity and HT were higher in the southern African region compared to other regions for both sexes (Gómez-Olivé et al., 2017). In African populations, the likelihood of being hypertensive in obese individuals increased with age similar to previous reports in the Chinese population (Zhang et al., 2019). The link between HT and ethnicity requires further investigation but is complicated in admixed populations (e.g. African Americans) and racial/ethnic descriptors are difficult to define accurately. Because of this challenge, associations between risk factors and diseases (like HT) are often related to ethnicity when they are in fact the result of a combination of culture, genetics, diet and lifestyles common to a particular group of people. Genetic ancestry alone can therefore not explain the differential prevalence and epidemiology of HT across different populations. In fact, it is well reported that HT prevalence and epidemiology differ significantly between populations with shared genetic ancestry but differing cultures, lifestyles and diets (Zhou et al., 2021).

1.2.2.6 Sex

Sex differences play an important role in the risk, mechanisms and outcomes of HT. Worldwide trends suggest that undiagnosed and untreated HT are more prevalent in men compared to women (Zhou et al., 2021). Cross-sectional studies have recently shown that sex differences in HT prevalence vary by age, such that at older ages, uncontrolled HT is more common in women compared to men (Daugherty et al., 2011; Gillis and Sullivan, 2016; Bager et al., 2023; Yeo et al., 2024).

The modulation of sex-related HT is complex. In women, RAAS elements are influenced by oestradiol and fluctuate in response to the changing levels of oestradiol throughout the menstrual cycle (Chidambaram et al., 2002). This is also supported by genomic data, with oestrogen receptor genetic variants found to be associated with salt-sensitive HT (Manosroi et al., 2017). The hormonal changes which occur during menopause therefore potentially contribute to the age-related differences in HT prevalence observed in pre- compared to

post-menopausal women (Maas and Franke, 2009), and when comparing men to women. Interestingly, studies investigating the potential determinants of age-related sex differences in HT, found that clinical characteristics such diabetes, BMI and kidney function did not differ between men and women with age (Yeo et al., 2024). The current literature therefore suggests that hormonal differences drive the observed age-related sex differences in HT.

1.2.2.7 HIV/AIDS

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) is now recognized as a major contributor to HT, especially in Africa, as populations on the continent carry the largest burden of the disease (Dwyer-Lindgren et al., 2019). A meta-analysis of worldwide data demonstrated that HT is more prevalent in HIV-infected individuals compared to HIV-uninfected individuals (Xu et al., 2017a). The pathophysiologic mechanisms of HT in people living with HIV is not well understood although believed to be multifactorial. HIV maintains the immune system in a chronic state of activation, thus promoting endothelial dysfunction, arterial stiffness and vascular damage; which are all contributors to increased BP (Masenga et al., 2019). Antiretroviral (ARV) therapy, although lifesaving, also contributes to HT in people living with HIV. Some ARVs, such as protease inhibitors, have been reported to increase the incidence of weight gain leading to metabolic syndrome and insulin resistance (Bakal et al., 2018; Sax et al., 2019; Mukuna et al., 2024). This potentially increases the risk of secondary HT. HIV infection and ARV therapy have been reported to impair renal function (Melendez Rivera and Hashmi, 2025), which may result in disrupted sodium regulation and an increase in BP.

1.3 Hypertension treatment

The section above emphasizes that the aetiology of HT is complex and involves interactions between many modifiable and non-modifiable factors as well as physiological pathways for controlling blood pressure. All these contributors should therefore be considered when developing strategies for treating HT. The goal of treatment for HT is to reduce systolic and diastolic blood pressure, to minimise associated disease risk. For a HT intervention to be effective it needs to target pathways which regulate blood pressure or modify the risk factors associated with HT.

1.3.1 Lifestyle changes

Lifestyle changes are a vital part of preventing the onset of HT and reducing the risk of cardiovascular diseases. Lifestyle changes target the modifiable risk factors of HT such as obesity, sodium and potassium intake, consumption of alcohol, use of tobacco and sedentary lifestyle (Dickey and Janick, 2001). In the largest clinical trial assessing the impact of weight loss on blood pressure, the amount of weight loss could be used to estimate the extend of blood pressure reduction (Whelton et al., 1992). Many more studies have shown that weight loss through physical activity and dietary modifications, throughout all stages of life from childhood to old age, reduces systolic and diastolic blood pressure (Wassertheil-Smoller et al., 1992; Whelton et al., 1998; Ho et al., 2016; Hagman et al., 2019; Schenkelaars et al., 2021). Smoking cessation and reducing alcohol intake have also shown to lower blood pressure, although studies are scarce (Roerecke et al., 2017; Gaya et al., 2024). Lifestyle changes directed at HT management also have a financial impact on health systems by potentially

lessening the need for pharmacological interventions (Dickey and Janick, 2001). It is unknown whether long-term adherence to lifestyle changes is better than adherence to pharmacological interventions in hypertensives. However, no studies have reported adverse effects related to lifestyle changes, which is a common contributor to poor adherence to pharmacological interventions (Munger, 2000).

1.3.2 Diuretics

Diuretics, more specifically, thiazides and thiazide-like diuretics, have been the recommended first-line medication of choice for treating HT (James et al., 2014). Diuretics are a class of drugs that affect renal fluid regulation, increasing water and electrolyte secretion(Wile, 2012), thereby increasing the volume and production of urine. The different types of diuretics, each with their own mechanism of action and each best suited for treating different conditions, are discussed below.

1.3.2.1 Carbonic Anhydrase Inhibitors

The mechanism of action for this group of medications is non-competitive disruption of the binding of H+ and $HCO3^{+}$ ions to carbonic anhydrase enzymes in the luminal side of the Bowman's capsule (Arumugham and Shahin, 2023). The prevention of ion reabsorption also prevents water reabsorption in the renal tubule. Acetazolamide is the most popular diuretic in this group. Acetazolamide is primarily used to treat heart failure as it increases renal perfusion and prevents fluid build-up when the heart is pumping inefficiently (Farzam and Abdullah, 2024). However, its mechanism of action of reducing $HCO3^{+}$ ions has a downstream effect of increased expiration of CO^2 and inspiration of O^2 through hyperventilation, which prevents tissue hypoxia at high altitudes and makes it effective for treating altitude sickness (Farzam and Abdullah, 2024).

1.3.2.2 Loop diuretics

Medications in this group act in the thick ascending limb of the Loop of Henle section of the kidney and target the electroneutral Na^{+}-K^{+}-$2Cl^{-}$ cotransporter 2 (NKCC2) which is responsible for the reabsorption of Na^{+} ions down a gradient into the cells (Mount, 2014). This pathway is main mechanism of urinary concentration in the kidney (Mount, 2014). Loop diuretics are anions which block NKCC2 channels, preventing Na^{+} movement and consequently water reabsorption (Hampel et al., 2018). Furosemide is the most commonly used loop diuretic to treat heart failure-associated fluid build-up, especially in patients with chronic kidney disease (Arumugham and Shahin, 2023).

1.3.2.3 Potassium-sparing diuretics

Medications in this group act in the collecting duct of the nephron which is responsible for up to 5% of sodium reabsorption, primarily through aldosterone and epithelial Na^{+} channels (ENaC) (Palmer and Schnermann, 2015). ENaC inhibitor Amiloride and aldosterone receptor antagonist Spironolactone are the most commonly used drugs in the class to treat the side effects of other more potent diuretics, antibiotics, chemotherapeutic drugs and immunosuppressants (Arumugham and Shahin, 2023).

1.3.2.4 Thiazide diuretics

Medications in this group act in late distal convoluted tubule where up to 10% of Na^+ ions are reabsorbed through the NaCl co-transporter (NCC) (McCormick and Ellison, 2015). Thiazide-diuretics act by competing with chlorine ions for the binding site on the transmembrane domain of NCC thereby preventing Na^+ (and in turn, water) reabsorption (Arumugham and Shahin, 2023). Thiazide diuretics are the superior diuretics for treating HT with the least adverse effects (Liang et al., 2017) (Figure 1).

Hydrochlorothiazide (HCTZ), chlorthalidone and Indapamide are the most used drugs in this class. The antihypertensive effects of thiazide diuretics include increased sodium excretions and decreased extracellular volume, both of which reduce cardiac output (Arumugham and Shahin, 2023). Thiazide diuretics also have long-term antihypertensive effects through inhibition of the sympathetic, and renin-angiotensin aldosterone systems, resulting in decreased vascular resistance (Wile, 2012).

Low dose (up to 50 mg) thiazide diuretics are considered most effective for treating HT, whereas high dose (up to 100 mg) are considered most effective for treating chronic heart failure (Arumugham and Shahin, 2023). HCTZ is orally administered, is 70% bioavailable and does not undergo any metabolism prior to being excreted in urine and faeces (Welling, 1986). Hyponatremia, hypo- and hyperkalaemia, hypomagnesemia and hyperuricemia are serious but uncommon side effects of thiazide-diuretics which have been previously reported (Sica, 2007). The more concerning side effects which have been reported with diuretics include hyperglycaemia and hyperlipidaemia. Prolonged thiazide diuretic therapy has been linked with glucose intolerance and diabetes mellitus onset (Furman, 1981; Sowers and Bakris, 2000; The ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, 2002; Verdecchia et al., 2004; Zhang and Zhao, 2015; Hall et al., 2020; Afify et al., 2023). However, the findings are confounded by inconsistent definitions of diabetes, small sample sizes, limited follow ups and incomparable study designs and therefore their interpretations can be challenged. Furthermore, a large, prospective study non-diabetic adults on diuretic HT treatment concluded that diuretics do not increase the risk of developing diabetes (Gress et al., 2000). It is also worth noting that the doses of HCTZ were not reported, which is a major limitation to the study. Short-term diuretic therapeutic use has been linked with a modest increase in low-density lipoproteins and triglycerides in black patients, males, diabetics and non-responders (Kasiske et al., 1995; Lakshman et al., 1999; Mantel-Teeuwisse et al., 2001). This link was observed when high doses of diuretics were administered and, where longitudinal data were available, the lipoprotein and triglyceride levels returned to baseline levels. This side effect seems to be temporary and only seen when high doses are administered.

Thiazide diuretics, specifically HCTZ and chlorthalidone, are the most commonly prescribed HT medications due to their effectiveness, low cost and long-term safety. Thiazide diuretics are the most cost-effective HT treatment prescribed in the United States (Park et al., 2020). In sub-Saharan Africa, HCTZ had the lowest median price and is the least variably priced HT medication (Twagirumukiza et al., 2010). Thiazide diuretics are also popular as part of combination therapy as they alleviate the water/sodium retention effects of other antihypertension medications (Skolnik et al., 2000).

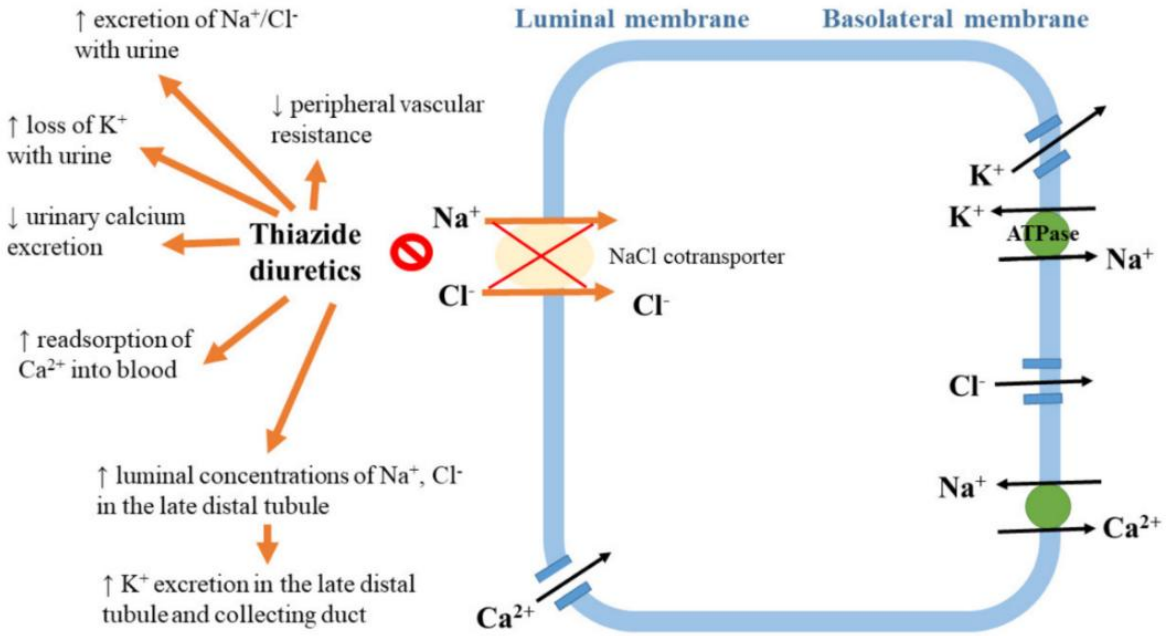


Figure 1.3. 1: Physiological effects of thiazide diuretics in the distal convoluted tubule region of the kidney (Rysz et al., 2020).

1.3.3 Calcium Channel Blockers

Calcium channel blockers (CCB) or calcium channel antagonists are a drug class widely used to treat various vascular conditions. CCBs act in the heart, vascular tissue and pancreas by binding to the L-type voltage-gated calcium channels and inhibiting the movement of calcium ions (Lin and Ma, 2018) (Figure 2). CCB activity results in vascular muscle relaxation and decreased vascular resistance, which in turn reduces arterial blood pressure (Eisenberg et al., 2004).

CCBs are grouped into two major categories: dihydropyridines and non-dihydropyridines (McKeever et al., 2024), depending on their binding locations. Non-dihydropyridines such as verapamil and diltiazem, act on the sinoatrial and atrioventricular nodes, slowing cardiac conductivity (McKeever et al., 2024). Non-dihydropyridine CCBs are therefore ideal for treating HT, tachycardia, atrial fibrillations and chronic stable angina (McKeever et al., 2024). Dihydropyridines such as amlodipine and nifedipine, act on the myocardium making them potent vasodilators (Eisenberg et al., 2004). This makes dihydropyridines ideal for treating HT and migraines (McKeever et al., 2024). CCBs are administered orally but have low bioavailability as they are metabolised by CYP3A4 enzymes in the liver (Reid et al., 1988).

Distribution characteristics for CCBs include being highly protein bound and have high volumes of distribution (Reid et al., 1988). Excretion of CCBs is primarily through urine (Reid et al., 1988). Unfortunately, medications in this group contribute greatly to drug-related fatalities. Because CCBs are hepatically metabolized by CYP3A4, once the enzyme is saturated, circulating concentrations can quickly become lethal resulting in severe adverse effects (McKeever et al., 2024). Additionally, CCBs interact with many drugs also metabolized by CYP3A4 and may cause adverse effects in that way (McKeever et al., 2024). Hypotension, bradycardia and blocked insulin production are primary symptoms of CCB overdose, followed by fatigue, confusion, light-headedness, coma and even sudden death (Alshaya et al., 2022). Administering high doses of CCBs is not recommended and patients initialized on CCBs require close monitoring in the initial stages (Alshaya et al., 2022).

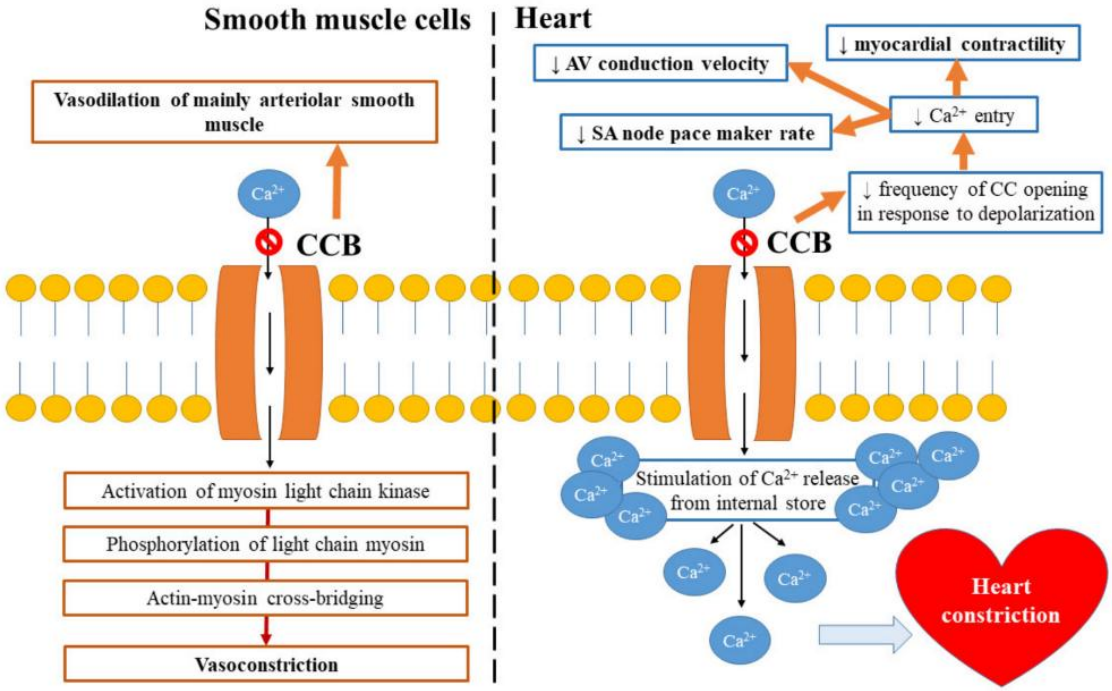


Figure 1.3. 2: Site of action and targets of calcium channel blocker (CCB) mechanism of action (Rysz et al., 2020)

1.3.4 RAAS blocking agents

The Renin-Angiotensin-Aldosterone system is primarily responsible for fluid, blood pressure and sodium homeostasis through mechanisms in the kidney, central nervous system and cardiovascular system (Sparks et al., 2014). Targeting this pathway is therefore a successful strategy in the treatment of HT, chronic kidney disease and congestive heart failure (Herman et al., 2024a; Hill and Vaidya, 2024). Angiotensin II receptor blockers (ARB) and ACE inhibitors (ACEi) are the main drug classes which target this pathway (Figure 3). Angiotensin II is the main vasoactive component of the RAAS and acts on Angiotensin I and angiotensin II receptors (Hill and Vaidya, 2024). Angiotensin II activity induces sodium and water retention, increases sympathetic nervous system activity, increases vascular resistance, and increases contraction of vascular smooth muscle, all of which increase blood pressure (Cernes et al., 2011). ARBs block the action of angiotensin II on the angiotensin receptors. Candesartan (up

to 32mg) and Losartan (up to 50mg) are the most commonly used ARBs (Hill and Vaidya, 2024). ARBs are well tolerated and adverse effects have been rarely reported (Rodgers and Patterson, 2001). However, ARBs may increase the effects of other antihypertension agents when administered together, resulting in hypotension (Hill and Vaidya, 2024).

ACEi acts through blocking the conversion of angiotensin I to angiotensin II by angiotensin converting enzyme (Herman et al., 2024a). The exact biochemical and physiological mechanism of action of ACEi are not known, however ACEi reduce blood pressure by inducing vasodilation and preventing sodium and water retention (Timmermans et al., 1993). Enalapril, Captopril and Fosinopril are the most commonly used ACEi medications and are administered orally (although Enalapril can be administered intravenously (Herman et al., 2024a). Multiple adverse effects have been linked to ACEi administration. The most common adverse effect is the development of a cough (Pinargote et al., 2014). ACEi have also been reported to significantly cause angioedema (swelling), especially in African Americans (Slater et al., 1988). ACEi have also been reported to cause acute reversible renal function decline (Murphy et al., 1984). ARBs are often recommended as an alternative to patients who experience ACEi adverse effects (Hill and Vaidya, 2024).

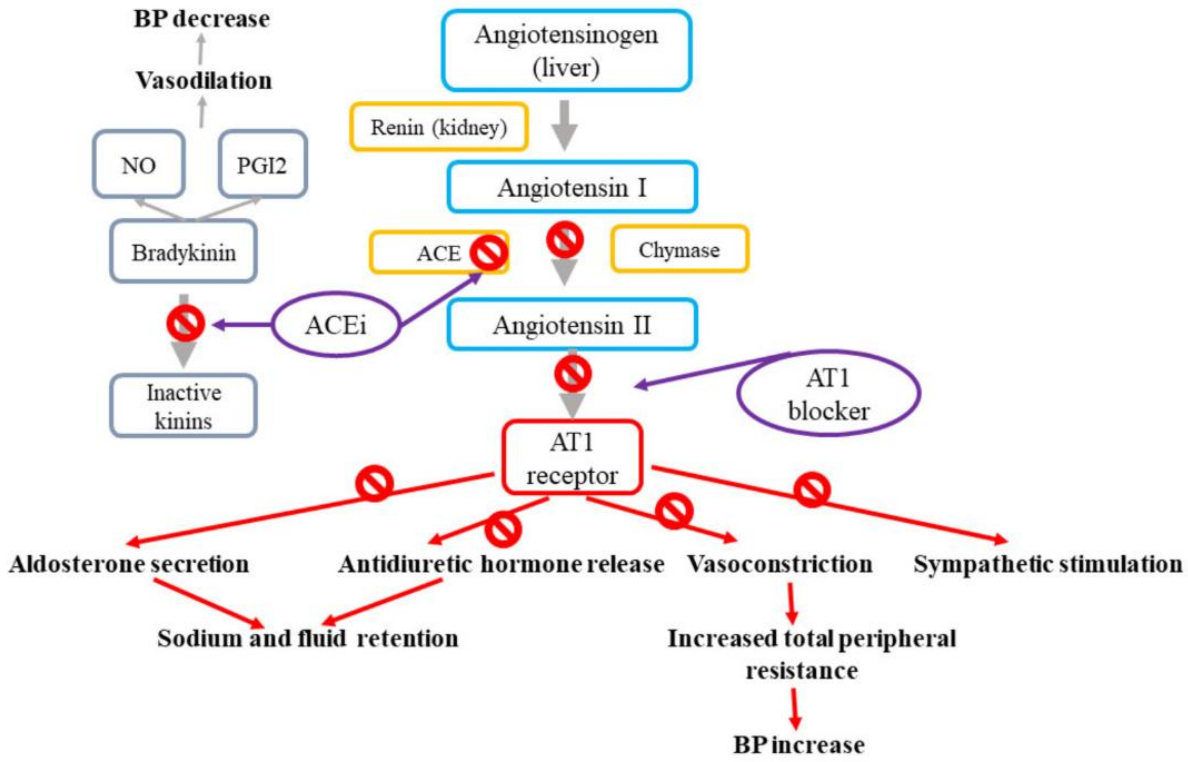


Figure 1.3. 3: Site and mechanism of action RAAS targeting drug classes, Angiotensin II receptor blocker and ACE inhibitors (Rysz et al., 2020).

1.3.5 Alpha/Beta-Adrenergic blockers

Alpha/Beta Adrenergic blockers (α/β-blockers) are a class of drugs widely prescribed for treating HT, although not as first-line therapy. α/β-blockers are primarily used to treat cardiovascular conditions such as coronary heart disease, cardiac arrhythmias, congestive

heart failure, myocardial infarction and tachycardia (Farzam and Jan, 2024). α/β-blockers bind to the alpha and beta receptors of catecholamine neurotransmitters and induce different effects in line with which organ the blocking occurs in (Farzam and Jan, 2024). Atenolol, bisoprolol and metoprolol selectively bind to beta-1 receptors in the heart, making them preferred for treating cardiovascular conditions including HT (Gorre and Vandekerckhove, 2010). Blocking of beta-1 receptors reduces renin release and cardiac output thus lowering blood pressure (Farzam and Jan, 2024). α/β-blockers are administered orally and have a short half-life (Gorre and Vandekerckhove, 2010). Alpha and beta receptors are present everywhere in the body and therefore many adverse effects have been reported with α/β-blocker treatment. These include dizziness, fatigue, constipation and nausea but sexual dysfunction, insomnia and weight gain have also been reported (Farzam and Jan, 2024).

1.3.6 Population-related differential HT treatment response

There is evidence that different populations respond differently to certain antihypertension medications. Investigations into this relationship date as far back as the late 20th century. Before the emergence of genetic studies, these differential treatment outcomes were believed to be driven by race (Gillum, 1979; Voors et al., 1979). The population descriptor "race" as used in these early studies is no longer considered accurate and therefore in this section population descriptors referring to ancestry will be adopted. Studies showed that differential treatment outcomes could be attributed to these differences in individuals of African and European ancestry and these ancestry-related effects could be reduced through combinations of different medications (Veterans Administration Co-operative Study Group on Antihypertensive Agents, 1982). At that time, most studies compared the differences in treatment response between self-reported "white" and "black" individuals, usually from the USA (Saunders et al., 1990) without reference to genetic ancestry. The pharmacokinetics (plasma concentration and half-life) of Nifedipine (a calcium channel blocker) in Nigerian individuals were found to be significantly different compared to that of "Caucasians" (individuals with European ancestry) (Sowunmi et al., 1995). Interest in this field coincided with the discovery that salt-sensitivity was heritable in "black" (modernly referred to as populations with African Ancestry) populations (Svetkey et al., 1996). Studies were therefore focused on the effect of this heritable salt-sensitivity on treatment response to different anti-hypertension medications (Weir et al., 1997). A study showed that controlling for salt-sensitivity through diet potentially removes race-related differences in treatment response (Weir et al., 1998). A meta-analysis of studies investigating the efficacy of different medications in "black" individuals confirmed that there were indeed differences in treatment response between these individuals and "white" individuals but the predictors remained unknown (Brewster et al., 2004).

Several large clinical trials have since confirmed that certain classes of antihypertension medications are more effective in certain populations compared to others (Gu et al., 2017; Flack and Buhnerkempe, 2022). As a result, the latest International Society of Hypertension guidelines recommend single pill combination with thiazide-diuretic and calcium channel blocker (Unger et al., 2020) for individuals with African ancestry. This recommendation is similar in European (McEvoy et al., 2024) and American (Whelton et al., 2018) HT guidelines. A large multi-site clinical trial in eight African countries has also shown that combination

therapy with a calcium channel blocker and HCTZ is most effective for African individuals (Ojji et al., 2019, 2022). The mechanisms behind this differential response in Africans and individuals with African ancestry are not well understood. It is hypothesized (and supported by some evidence) that the differential response is because HT in this group of individuals is driven by salt sensitivity compared to renin activity (Faconti et al., 2020).

Race and gender were suggested to only explain 11% (for systolic BP) and 4% (for diastolic BP) of inter-individual variation in response to antihypertension medication (Chapman et al., 2002) suggesting that other factors may be responsible for the observed race- and gender-related differences. The development of genetic analysis technologies has allowed for a new lens with which to understand the population differences in response to antihypertension treatment. This is important, now more than ever before, in populations with individuals with African ancestry as these individuals are disproportionally affected by HT mortality and morbidity.

1.4 Pharmacogenomics

Pharmacogenetics is "the study of variability in drug response due to heredity" (Nebert, 1999) and adding the suffix "omics" broadens the term to include all genes in the genome related to drug response (Pirmohamed, 2001). Pharmacogenomics is part of the field of precision medicine, which intersects multiple disciplines including pharmacology, molecular biology, physiology, human genetics, genomics and bioinformatics. Pharmacogenomic studies aim to understand the fundamentals of gene-drug relationships; from genetic variation to physio-pharmacological effects through molecular mechanisms (Evans and Relling, 1999).

1.4.1 Brief history of pharmacogenomics

The history of pharmacogenomics goes as far back as the 6th century, when Greek philosopher Pythagoras of Samos noted that some individuals were endangered by fava beans (later discovered to be glucose-6-phosphate dehydrogenase deficiency (Alving et al., 1956)). Although not directly related to drug response, the discovery of the laws of heredity by Gregor Mendel (Mendel, 1866), was pivotal to the eventual birth of pharmacogenomics. There were many isolated discoveries of heritable defects of metabolism throughout the late 19th and early 20th centuries, with Friedrich Vogel first coining the term pharmacogenetics in 1957 (Vogel, 1959). The field of pharmacogenomics was mostly driven by the discovery of polymorphisms in drug metabolizing enzymes during late 20th century. The most notable was the discovery of polymorphisms in affecting debrisoquine (an antihypertensive medication) metabolism (Mahgoub et al., 1977). Debrisoquine hydroxylase is now known as the drug metabolizing enzyme – cytochrome P450 2D6. The year 2000 was the most important year in the history of pharmacogenomics. In this year, the complete human genome was first drafted (Lander et al., 2001; Venter et al., 2001), resulting in the formation of the International SNP Map Working Group – a map of human genome sequence variation containing over 1.4 million single nucleotide polymorphisms (SNPs) (Sachidanandam et al., 2001). Currently, the field focuses on using advancing technology to characterize the different types of genetic variation (SNPs and structural variants) and ascertain their effect of drug metabolism and efficacy.

1.4.2 Absorption-Distribution-Metabolism-Excretion Gene variation and drug response

Foreign substances ingested into the body goes through a process which determines what the body does to the drug and what the drug does to the body. This is known as the absorption-distribution-metabolism-excretion (ADME) process. Absorption describes the movement of the drug from the site of administration to the site of action, distribution describes how the drug moves through the body, metabolism describes how the drug is broken down into its metabolites and excretion describes how the drug and its metabolites are removed from the body. The study of this process and drugs is known as pharmacokinetics. As this process occurs, drugs can cause physiological and biochemical changes in the body, and the study of this phenomenon is known as pharmacodynamics. Pharmacokinetics and pharmacodynamics are driven by proteins such as enzymes, transporters and receptors. Variation in the genes which encode these proteins may alter the pharmacokinetics/dynamics of a drug, and lead to variable drug response and adverse effects. Genes in the ADME process which contribute to variable drug response are known as pharmacogenes.

SNPs and copy number variants are the most common and researched types of genetic variation in pharmacogenes. These genetic variations often have four types of effects on drug metabolizing enzymes: complete loss of function of the protein, reduced activity of the protein, increased activity of the protein, and extremely high amounts of the protein (due to copy number variation). The phenotypic classifications of these effects are poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM) and ultrarapid metabolizer (UM). Cytochrome P450 enzymes are responsible for metabolizing over 70% of all therapeutic drugs (Lynch and Price, 2007).

Traditionally, cytochrome P450 enzymes have been emphasized in pharmacogenomics, as non-optimum enzyme function due to mutant alleles can lead to inefficacy (if the drug is not activated), or adverse effects (if the drug is metabolized too quickly). Cytochrome P450 enzymes are highly polymorphic and the allele frequencies of variants in these genes differ significantly across world populations (Masimirembwa and Hasler, 2013). For example, a genotype which results in a PM phenotype may be more present in one population and absent in another. The same drug can therefore have different effects in different populations. Pharmacogenomics and drug monitoring can thus be combined in drug trials to optimize treatment for different populations. Variation in transporter proteins is an emerging area in the field of pharmacogenomics. Variation can influence drug response by altering the amount of drug in the blood and subsequently available to target tissues (Vasiliou et al., 2009; Ahmed et al., 2016).

1.4.3 Pharmacogenomics in Africa

The development and adaptation of pharmacogenomic tools and practices lags behind in African populations, similar to most developing populations around the world. An increase in funding and scientific skills capacity has largely increased the advancement of pharmacogenomics in Africa. Studies have shown that in Africa, there is greater inter-population diversity in pharmacogene variation compared to populations on other continents (Dandara and Matimba, 2019) and there are many gaps in knowledge regarding how the

pharmacogene-variant frequencies in African populations compare to the frequencies in populations wherein associations have been previously reported. Furthermore, studies have shown substantial geographical differences in the distribution of genetic variation of known ADME genes (Rajman et al., 2017). This suggests that there is a need for extensive characterization of African genomes, for a better understanding of pharmacogenomic variation in this population.

Although most pharmacogenomic studies in Africa have focused on searching for African population-specific ADME genotypes, there has also been a concerted effort to investigate how pharmacogenomics can contribute to the management of diseases such as HIV/AIDS, Malaria and Tuberculosis which disproportionally affect the continent. HIV/AIDS has been a major public health problem in sub-Saharan Africa for the past 2 decades (Danel et al., 2015), motivating the development and approval of many drugs to deal with endemic (Gandhi et al., 2023). The chronic nature of the disease means that continued treatment adherence is essential to reduce new infections and improve the quality of life of those living with HIV/AIDS (Mills et al., 2006). Side effects are one of the most common reasons cited for treatment non-adherence (Almeida et al., 2024). CYP2B6, responsible for the metabolism of two key antiretroviral medications, efavirenz and nevirapine, is often implicated in adverse drug reactions and differential drug response (Ward et al., 2003; Penzak et al., 2007). Deficient alleles CYB2B6*18 (an isoleucine-threonine substitution at position 328) and CYP2B6*6 (a haplotype of glutamine-histidine substitution at position 172 and lysine-arginine substitution at position 262) are well studied and have been shown to be highly prevalent and associated with high plasma concentrations of efavirenz and nevirapine, in the sub-Saharan African population (Ngaimisi et al., 2013; Mukonzo et al., 2014; Mhandire et al., 2015). The high prevalence and biological impact of these alleles in African populations means that these populations are susceptible to side effects such as neuropsychological toxicity (Mukonzo et al., 2014) and epidermal necrolysis (Carr et al., 2014). This example highlights the need for tailored dosing for African HIV/AIDS patients, guided by pharmacogenomic studies on the genotypes unique to the population. Such studies have shown that lowering the dose of efavirenz for African patients with HIV/AIDS may still achieve the desired therapeutic response and minimize side effects (Mukonzo et al., 2014).

Although there are currently no clinical implementations of pharmacogenomics in the treatment of HT, a good case study for the potential of pharmacogenomics in African populations is *CYP2D6* and metoprolol. *CYP2D6* metabolizes up to 25% of all registered drugs (Zanger and Schwab, 2013) including metoprolol, a drug primarily used to treat hypertension, arrhythmias and heart failure (Morris et al., 2025). The pharmacokinetics of metoprolol are therefore substantially affected by the metaboliser status of the CYP2D6 enzyme. Poor metabolizer phenotypes have been associated with greater response to metoprolol compared to the other CYP2D6 phenotypes (Meloche et al., 2020). Several guidelines therefore recommend different doses for individuals with different CYP2D6 phenotypes, including African individuals (Duarte et al., 2024; Whirl-Carrillo et al., 2021).

1.5 Genetic contribution to HT treatment response

The link between genetics and HT itself has been vastly studied. Various studies have shown that blood pressure variation is 30-50% heritable (Padmanabhan et al., 2012; Ehret and Caulfield, 2013; Munroe et al., 2013; Hiltunen et al., 2015). However, much work has yet to be done to identify genetic differences between different treatment outcome phenotypes and the pathways which drive them.

The two most common approaches used to identify genetic variants associated with treatment response are candidate gene and genome-wide association studies (GWAS). Candidate gene studies are hypothesis-driven, focusing on specific genes involved in a particular trait or disease leveraging prior knowledge or previous studies (Amos et al., 2010). The candidate gene approach is limited to a select few genes of interest and its methodologies include testing for associations between the trait and variants in selected genes. This approach has the major advantage of being faster and much cheaper than GWAS as fewer genes and variants are analysed. Candidate gene studies often have immediately evident biological relevance and interpretable results, as the candidate genes (if selected well) will have known function related to the trait/disease (Zondervan and Cardon, 2007). Unfortunately, the accuracy of the assumptions that are made to test the hypothesis, determine the success of the study. If the selected genes are not related to the trait/disease, the relationships of interest will be missed. Another disadvantage of this approach is that it has limited ability to discover variants in other genes which have not been previously associated with the trait/disease (Amos et al., 2010).

GWAS differs from the candidate gene approach as it does not rely on prior knowledge and assumptions about genes but instead scans the entire genome for variants which are statistically associated with the trait/disease. GWAS are hypothesis-free, and typically compare the frequencies of millions of variants in individuals with the trait/disease vs individuals without, to find associations (Zondervan and Cardon, 2007). The biggest advantage of GWAS are their discovery potential, by comprehensively scanning the entire genome, previously unknown variants and biological pathways can be discovered (Zondervan and Cardon, 2007). GWAS are especially powerful for complex diseases such as HT, as they are suited to find multiple risk genetic loci associated with treatment response. These successful associations can be used to identify predictive biomarkers, integrated into personalized treatment plans and incorporated into clinical trial design. The disadvantage of this approach is that analysing the entire genome requires costly and intense resources to generate and analyse data (Amos et al., 2010). Furthermore, GWAS are likely to find small effect size variants which require large sample sizes for statistical power. Since much of the human genome is non-coding, identified variants may not have immediately understandable biological functions, making interpretation very challenging (Zondervan and Cardon, 2007).

Table 1. 2: Selected examples of genes associated with HT treatment efficacy and with potential for guided therapy

Gene	Variant	Protein	Biological consequence
Thiazide diuretics			
NEDD4L	rs4149601 (G/A)	4-like, E3 ubiquitin protein ligase	Cryptic splice site and better response
PRKCA	rs16960228 (A/G)	Protein kinase C alpha	Intronic variant and better response
Beta-blockers			
ADRB1	rs1801252 (Ser49Gly), rs1801253 (Arg389Gly)	β1-adrenergic receptor	Changes in receptor sensitivity and greater BP and heart rate lowering
RAAS inhibitors			
ACE	rs1799752 (I/D)	Angiotensin Converting Enzyme	Increased enzyme activity and better response
Calcium Channel Blockers			
CACNA1C	rs312481 (G/A)	Voltage-dependent calcium channel	Intronic variant and better response

Both the candidate gene approach and GWAS have been used for investigation the pharmacogenetics of HT treatment response (Oliveira-Paula et al., 2019; Rysz et al., 2020). The combination of candidate gene and GWAS has exponentially advanced our understanding of genetic influences on antihypertensive response, by identifying variants in over 30 genes involved in multiple blood pressure regulating pathways (Oliveira-Paula et al., 2019; Rysz et al., 2020). These studies have included a wide range of ethnic groups, which has deepened our understanding of gene-environment interactions and population-level variability. These studies have investigated genetic associations with drugs from multiple classes, including angiotensin receptor blockers, ACE inhibitors, calcium channel blockers, beta-blockers and diuretics. This literature has been comprehensively reviewed by Rysz (2020), Katsukunya (2023) and Mabhida (2021) with many non-ADME genes shown to influence treatment outcomes.

The current available literature highlights several major limitations in our knowledge of the extent to which genetics influences treatment response, the mechanisms and the clinical utility of this relationship. The complicated nature of HT means that there is heterogeneity in antihypertension response phenotypes. Treatment response is often described as reduction in systolic and diastolic blood pressure after treatment is administered. However, different study designs make it difficult to compare across studies. For example, both Svensson-Färbom et. al and Mcdonough et. al reported that G-allele carriers of rs4149601 had greater reduction in systolic and diastolic blood pressure in response to HCTZ treatment (2011; 2013a).

However, Svensson-Färbom et. al (2011) genotyped participants recruited from two different studies with different follow up periods (3 weeks vs 24 months) and this was different to Mcdonough et. al (2013a), who used a follow up period of 6 months. The temporal effect on blood pressure reduction is unaccounted for and therefore although both studies somewhat reported the same association, they do not support each other. Studies in the current literature also report contradictory outcomes. Sciarrone et. al reported that individuals with the I-allele have a better response to HCTZ in Caucasians whereas Schelleman et. al reported that the I-allele was not associated with better response to diuretics in Caucasians (2003; 2006). Another limitation of the current literature is that it does not contain information which is crucial for implementing precision medicine. This information includes demographic and lifestyle information, environmental exposure, adherence laboratory measurements and longitudinal data (Lim et al., 2024).

Some studies report reductions in systolic and diastolic blood pressure whereas others report the occurrence of adverse effects (Oliveira-Paula et al., 2019). Many studies, especially the candidate gene studies, had small samples sizes limiting the statistical power to detect significant associations. This was similar for GWAS, as no reported study to date has amassed 6 000 individuals (Rysz et al., 2020), which is a moderate sample size for GWAS (Zondervan and Cardon, 2007). The current literature has also been single-locus focused, overlooking the impact of gene-gene interactions and epistatic effects on antihypertension response. Perhaps, the biggest limitation of the current literature on the genetics of HT treatment response is the underrepresentation of continental African populations. There is only a single candidate gene study, conducted in South Africa which investigated the frequencies and associations of previously reported variants in linguistic-ethnic groups (Masilela et al., 2020). 291 hypertensive individuals on chronic care from the Xhosa, Swati and Zulu tribes were genotyped using MassArray technology (Masilela et al., 2020). Alleles of rs2107614, rs2776546, rs2070744 and rs7297610, variants in the *WNK1, YEATS* and *ADRB2* genes, were significantly associated with uncontrolled blood pressure amongst the different tribes (Masilela et al., 2020). The small sample size and lack of longitudinal data were limitations in this study. Furthermore, adherence was not assessed in these patients which would play a major role in treatment response. Additionally, the study was limited to associations of treatment response with genetic variants previously reported from populations outside of Africa thus excluding any variants specific to continental Africans.

The need for GWAS and candidate gene studies in African populations, investigating the presence and contribution of genetic variants to treatment response is evident. This will potentially offer insights into the mechanisms with which thiazide-diuretics and calcium channel blockers have a greater efficacy in this population compared to RAAS blocking medications. Additionally, this will offer insight into whether the variants linked to differential response in European, American and Asian populations are present or have a similar effect in African populations.

The motivation for the work described in this thesis is the high burden of HT in South Africa and the evidence of differential treatment response in the population. The results of this work may provide evidence of novel variants, unique to Black South Africans, which contribute to

treatment response and may also add to the current knowledge of the link between genetics and treatment outcomes with the potential of improving efficacy through genetics-based precision medicine. This subgroup of individuals was chosen because due to historical context in South Africa, these individuals are comparable regarding environmental exposures such as lifestyle, diet and socio-economic status. Additionally, individuals in this subgroup often belong to tribes which are genetically comparable due to their connected ancestral history (Sengupta et al., 2021). Black South Africans are also severely understudied in general and underrepresented in genetic studies. The population description used does not align with the published recommendations for population descriptors, but is aligned to South African national common use (National Academies of Sciences, 2023). Therefore, given the context provided above, "Black South Africans" accurately and inclusively describes the group of individuals that were the focus of the study.

1.6 Study setting

This section outlines the study setting of this project. The specific details of methodologies and statistical analyses used are described in Chapters 2 and 3 in the format of manuscripts. The study was conducted in Soweto, Johannesburg, South Africa.

1.6.1 Soweto, Johannesburg, South Africa

Soweto, an acronym for *South Western Townships*, is a large urban area within the city of Johannesburg, in the Gauteng province of South Africa. The settlement was historically established in the early 20th century as a residential area for Black South Africans under apartheid laws (Bonner and Segal, 1998). It is one of the largest townships in South Africa, and holds substantial historical, cultural, and socio-economic significance. Soweto was a focal point of the anti-apartheid struggle, notably in the 1976 Soweto Uprising, which marked a pivotal moment in South Africa's fight against racial segregation and oppression.

Soweto is densely populated with a population of over 1.6 million people and covers approximately 200 km^2 of land. 80% of Soweto residents live in formal housing, with many living in government-built homes for low-income families. The remaining population resides in informal housing such as shacks and often lack basic services such as plumbing and electricity. Today, Soweto remains a vibrant community characterized by cultural diversity, resilience, and a strong sense of identity, but also faces considerable socio-economic challenges. Residents experience higher-than-average rates of poverty, unemployment, and limited access to healthcare and other resources. According to a national survey, only 9.3% of individuals above the age of 20 years had a tertiary education qualification, 55% of households had running water and 19% earned no income annually (Statistics South Africa | Census Dissemination, 2022).

The legacy of apartheid-era policies has led to persistent disparities in health, income, and education within the area. These factors make Soweto a compelling setting for studies in various fields, particularly public health and social sciences, as researchers can examine the interplay of socio-economic factors, historical context, and health outcomes. Soweto's unique context provides valuable insights into the health and social challenges faced by similar communities across South Africa and other parts of the world with histories of systemic inequality. We identified 7 out of 25 primary health clinics in Soweto as suitable for our study

due to their proximity to the site from which recruitment was managed, Perinatal Health Research Unit, Chris Hani Baragwanath Hospital Complex.

Historically, migrant workers from other provinces and countries immigrated into Soweto for employment (Richter et al., 2009). Soweto therefore contains a diverse genetic architecture developed through mixing of different ethnolinguistic groups as well as a historical Caucasian and Indian presence in the area. Although, southern African Bantu-speaking tribes are ancestrally similar (Sengupta et al., 2021), the genetic substructure in Soweto is unique compared to other well-studied Black African populations (May et al., 2013). Soweto therefore provides a valuable resource for conducting genetic research, as multiple ethnolinguistic groups are represented.

1.7 Study aim, objectives and overview of methods

The aim of the study was to build a prospective cohort of individuals newly diagnosed with HT and to assess the impact of genetic variation in candidate genes on the treatment outcomes of first-line HT treatment in Black South Africans in Soweto.

Objective 1: Observe and characterise newly diagnosed hypertensives from clinics in Soweto

This objective set out to build a cohort of newly diagnosed hypertensives and collect relevant information relating to the patients and their treatment.

Approach: We adopted an observational, longitudinal and prospective study design. Once informed consent was received, patients were recruited into the study and seen over multiple timepoints. Anthropometric, family history and treatment data were collected through a questionnaire. Participants were stratified based on their prescribed medications and their treatment response status at each visit. Non-parametric tests were used to check for differences in anthropometric measurements between individuals in different treatment outcome groups and linear mixed models were used to search for potential predictors of treatment response.

Longitudinal data introduces a dependency problem, where the frequency and timing of the measurements differed between each person, and this may have biased the outcome. In a longitudinal study design, multiple observations from the same person tend to be similar. The residuals (the difference between a value predicted by a model and the corresponding observed value) calculated for the model are therefore non-random, violating a fundamental assumption of general linear regression models (Kim, 2019). Linear mixed models are therefore better suited for these data as they can include fixed (the same for everyone) and random (varying across persons) effects into the model (Hoffman, 2015). There are multiple sources of variation to be accounted for in these data. Each person naturally has a higher/lower outcome compared to the cohort mean and their residuals will deviate from the mean in the same direction across time. This is addressed by adding a random intercept to the model, such that each person will have their own random intercept which deviates from cohort intercept. The second source of variation in a longitudinal study is the individual-specific effect of predictors on the outcome over time. Linear mixed models address by adding

a random slope to the model. This way, the effect of time and predictors on each individual's outcome is accounted for in the model.

The model likelihood can be used to assess the quality of a model. In this calculation, the likelihood estimation function goes through different combinations of model parameter values and calculates the likelihood of the observed outcomes for each set of parameters. The function outputs the model parameter value combinations that have the highest likelihood of producing the observed outcomes (and the parameter standard errors). The likelihoods of model parameter values for each observed outcome have to be multiplied together for the model, and this is mathematically unstable due to the cost of rounding off. Adding the natural logarithms of these likelihoods instead, mitigates this challenge. The log-likelihood is therefore an estimate of how well a model fits the data and was used in this analysis.

Unbalanced and non-symmetric data can result from some participants having more timepoints than others or if the time in between the timepoints is not the same for everyone. In order to overcome this limitation, the overall change in blood pressure between the first and second timepoints can be used as the outcome. The variable - time between timepoints, is then incorporated into the analyses as a predictor in anticipation that it would affect the outcome of each person in an unknown direction.

The insights obtained from the research conducted to achieve this objective were drafted into a manuscript and extensively discussed in chapter 3 of this thesis.

Objective 2: Candidate gene selection and panel design

The purpose of this objective was to identify a set of genes that had previously been implicated in the biochemical pathways of HCTZ action or response to HCTZ in patients. Genetic variation in these genes would then be examined for association with HCTZ treatment outcomes.

Approach: PharmGKB is a resource which collects and curates available knowledge on the impact of genetic variants on drug response (Whirl-Carrillo et al., 2021) and was therefore the primary source of information when selecting the set of genes. PharmGKB contains a list of genes which have been investigated to play a role in the efficacy of HCTZ in treating HT. The raw data from PharmGKB contained unsorted information that required further screening and processing before selecting relevant genes. The filtering step included removing entries without gene names, where there was no association found, where the drug studied was not HCTZ and where the phenotype studied was toxicity and not efficacy (adverse effects were not a focus of the study). The prioritization step involved ordering the genes according to three metrics: supporting literature, sample size and p-values (of the associations in relevant studies). The results of this approach taken were entries grouped by gene and then ranked (within the group) by sample size and p-values. This approach was particularly useful because it provided insight into how significant the association of the genes was with response to HCTZ and allowed for the comparison of the sample sizes and findings of each study entry.

A custom targeted gene panel was designed using the DesignStudio tool. The DNA amplification primers were designed to target the exon, exon-intron boundaries and regulatory regions (5' and 3' untranslated regions) of the candidate genes in Table 1.2.

Table 1. 3: Candidate genes selected for the study and their genomic descriptions.

Gene	Build	Chrom	Start	End	Size(bp)	Strand[1]	Exon count	5'UTR (bp)	3'UTR (bp)
ADD1	Hg19	4	2,843,844	2,930,076	86,218	P	16	188	1,552
RAD52	Hg19	12	911,736	949,694	37,958	N	12	114	1,302
WNK1	Hg19	12	752,579	911,452	158,837	P	28	643	2,660
NEDD4L	Hg19	18	58,044,226	58,401,539	357,363	P	31	283	5,271
ACE	Hg19	17	63,477,061	63,498,380	21,320	P	25	34	1,014
AGTR1	Hg19	3	148,697,784	148,743,008	45,224	P	3	388	888
YEATS4	Hg19	12	69,359,710	69,390,870	31,160	P	7	221	480
NOS3	Hg19	7	150,688,144	150,711,687	23,544	P	27	296	430
PRKCA	Hg19	17	64,298,926	64,806,862	507,937	P	17	44	6,707
GNB3	Hg19	12	6,949,375	6,956,557	7,183	P	11	405	495
ALDH1A2	Hg19	15	58,245,622	58,358,121	112,500	N	13	273	1,773
TXNDC11	Hg19	16	11,772,943	11,836,648	63,706	N	12	127	112
TTC6	Hg19	14	38,064,834	38,311,922	247,089	P	33	475	426
SCNN1G	Hg19	16	23,194,066	23,228,204	34,139	P	13	113	1,414
HSD3B1	Hg19	1	120,049,833	120,057,667	7,845	4	P	138	409
ADO	Hg19	10	64,564,480	64,568,239	3,760	1	P	340	2,607

[1]P-Positive strand; N-Negative strand

Objective 3: Targeted next generation sequence, variant annotations and associations with response to HCTZ

This objective set out to identify genetic variants in the chosen HCTZ-related genes in Black South African hypertensives.

Approach: The Illumina ISEQ 100 (Illumina Inc, San Diego, California) platform was used for next generation sequencing (NGS) because it leverages the accuracy of sequencing-by-synthesis chemistry whilst utilizing a more affordable complementary metal-oxide semi-conductor technology. The ISEQ 100 platform has a max read length of 150 bases and paired-end sequencing capability. Each cartridge has capacity of sequencing up to 4 million reads and producing a max output of 1.2 gigabytes of data. Combined with the AmpliSeq Library Plus kit (Illumina Inc, San Diego, California) the total time required per run, from library prep to completed sequencing, was 3 days.

BWA-MEM algorithm was developed and is widely used for aligning sequenced reads to a reference genome (Li, 2013). BWA-MEM uses a seed-and-extend approach. First, the reference genome is indexed for fast and memory-efficient exact matching, using the Burrows-Wheeler Transform (BWT) algorithm (Li and Durbin, 2009). The algorithm then searches for the longest substrings of the sequencing read that match to the reference genome. These maximal exact matches (MEMs) serve as the seed for further alignment and are clustered to represent the most likely true alignment of the read region to the reference genome. As the alignment is extended around the most promising seeds mismatches, small insertions and deletions are handled and refined using the Smith-Waterman algorithm (Smith and Waterman, 1981). The final alignments are scored based on the quality of the match and number of mismatches.

Variants are identified by identifying the mismatched base pairs between the reference genome and the sequenced read. The workflow used to identify variants in this work was adapted using tools from the Genomic Analysis Toolkit (McKenna et al., 2010). In summary, a genome variant call file (gvcf) was created as an intermediate file to make joint calling of multiple samples easier. The tool HaplotyperCaller was used to find regions in the sequenced reads with SNPs and indels, identify possible haplotypes and calculate the likelihood of the haplotypes given the sequencing data. Single sample gvcfs were imported into a genomicsdb workspace in preparation for joint calling, using the tool GenomicsDBImport. Sequencing reads from each sample were then genotyped simultaneously using the tool GenotypeGVCFs. The identified variants were separated into SNPs and indels using the tool SelectVariants because filtering metrics differ for different types of variants. Raw variants were filtered using defined thresholds and the tool VariantFilteration. The alternative to this approach would be variant recalibration, which uses machine learning to identify good vs bad quality variants (DePristo et al., 2011). This approach requires high-quality known sets of variants for training and a lot of sequencing data. It was therefore less suited for the candidate gene analysis done in this work.

Once identified and filtered, variants were annotated in order to understand their possible biological impact. Several tools have been developed for clarifying variants, namely: Annovar,

SnpEff and Variant Effect Predictor (VEP). The tools incorporate information such as transcript set, gene ontology and sub-features to classify variants. The differences in this information between databases means that there is limited concordance in classifications across the tools (McCarthy et al., 2014). There is no evidence suggesting that any tool performs better than the others. VEP was used for variant annotation in this work as it returns tab-separated output with a consequence for each variant, for each transcript.

In silico prediction tools were used to gain insights on the functional impact of the variants identified. Different tools utilize different methods for detecting variants that are clinically relevant. This process involves using variants with known effects to validate variant predictions. Unfortunately, these tools have high false positive/negative results for African populations due to underrepresentation and high genetic diversity (Bope et al., 2019). This highlights the need for the development of prediction tools which take into account the genetic architecture of all populations. In this work, the "standard" prediction tools: CADD, REVEL, SIFT Indel, LOFTEE and SpliceAI, were used and their results compared.

Power calculation: The power for an estimated sample size of 200 case-control pairs was 99% to detect a risk allele with a 1.7 odds ratio and a minor allele frequency of 35% at a p-value of 0.05. The study sample size was appropriate to detect high effect variants that are common in the study group. Associations of the identified genetic variants with treatment outcome were assessed through a chi-squared test, testing whether the observed allele frequency (responders) of each variant is significantly different from the expected frequency (non-responders) under the hypothesis that there is no difference in allele frequencies between responders and non-responders.

The insights obtained from the research conducted to achieve objectives 2 and 3 were drafted into manuscripts and these are jointly contextualised and discussed in chapter 4.

1.7.1 Ethical clearance and certification

Informed consent preceded recruitment into the study. The University of the Witwatersrand, Human Research Ethics Committee (Medical) approved this PhD study with the clearance number: M220286, as a sub-study under the main study (M2011143).

Chapter 2: Hypertension treatment practices and outcomes at health community centres in urban Soweto, South Africa

This chapter contains the manuscript which set out to achieve objective 1. It describes the treatment practises and outcomes of newly diagnosed hypertensives treated at primary health centres in Soweto, South Africa. Although evidence-based treatment guidelines have been published by multiple international organizations, how these guidelines are implemented, especially in South Africa, has not been investigated. Understanding treatment practises and outcomes is the first step in identifying context-specific factors which influence hypertension management at primary health care level. The outcomes described in this chapter demonstrate that hypertension management at primary health care level is complicated and nuanced and that there are differential responses to treatment. This provides a valuable resource that can then be used for investigating the potential genetic contributions to this differential treatment response.

The work described in this chapter has been accepted for publication to the Cardiovascular Journal of Africa.

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MR, CM, ZW, NM, KN and AM were responsible for designing the study and refining the approach. KN carried out the study recruitment and data collection along with study nurses. KN and AM analysed the data. KN and DT drafted the manuscript. MR, CM and NM edited the manuscript, all authors reviewed and approved the submitted manuscript.

2.2 Abstract

In this study, we explored hypertension (HT) management in urban Soweto, South Africa, involving 364 participants from primary healthcare clinics. At baseline, 40% of participants were prescribed one drug, and 53% were given two drugs. Thiazide diuretic and calcium channel blocker were most prescribed at baseline. Blood pressure (BP) control at the first follow-up was achieved by 50% of those on single-drug therapy and 52% of those on two-drug therapy. Men showed higher rates of uncontrolled BP (60%) compared to women (42%). Significant factors influencing BP reduction included age, sex, waist circumference, and treatment clinic. Both single and combination therapies yielded similar outcomes in BP control. The study suggests that HT treatment approaches varied between clinics, potentially due to co-morbidities and continuity of care challenges. Further research is needed to identify best practices across different South African settings, considering drug availability and cost.

2.3 Introduction

Systemic arterial hypertension (HT), a condition characterized by systolic blood pressure (SBP) over 140 mmHg and diastolic BP (DBP) over 90 mmHg, is the leading modifiable risk factor for cardiovascular diseases (CVD), and the largest contributor to global mortality and burden of disease (WHO, 2023). Africa carries the largest burden of HT worldwide, with recent studies reporting prevalence as high as 48% (Akpa et al., 2020). A sub-study of the Africa Wits-INDEPTH partnership for Genomic Research (AWI-Gen) (Gómez-Olivé et al., 2017), which comprises population cohorts from multiple countries in sub-Saharan Africa (SSA), showed that South African study sites had the highest prevalence of HT compared to study sites in Burkina Faso, Kenya, and Ghana. Within South Africa, southeastern Bantu-speaking populations in Soweto had the highest prevalence of HT (54%) compared to those in Agincourt (Mpumalanga Province; 47%) and Dikgale (Limpopo Province; 41%) (Gómez-Olivé et al., 2017).

Hypertension has been emphasized as a primary modifiable risk factor for cardiovascular diseases and mortality within SSA (GBD 2017 Risk Factor Collaborators, 2018). It is a significant contributor to ischemic heart disease in the region, alongside risk factors like smoking, diabetes, abdominal obesity, and dyslipidemia (Mensah, 2008). Moreover, hypertension-related complications substantially contribute to hospital fatalities, especially among younger individuals. The increasing prevalence of hypertension at younger ages, combined with low rates of outpatient diagnosis, treatment, and blood pressure control, highlights the considerable challenges posed by hypertension in the region (Helelo et al., 2014). Additionally, the coexistence of hypertension with other health conditions, such as HIV infection, adds further complexity to managing cardiovascular risk factors in SSA (Xu et al., 2017b). Research indicates a significant proportion of people living with HIV (PLWH) also suffer from hypertension, stressing the need for integrated healthcare strategies addressing multiple risk factors concurrently (Xu et al., 2017b).

In response to the high rates of HT in SSA, the Southern African Hypertension Society released an evidence-based guideline for medical practitioners, nurse practitioners, and pharmacists, to aid in simplifying assessment and treatment (Seedat et al., 2014). Key aspects of this guideline included incorporating cardiovascular risk into the treatment strategy, clearly

defining the goals of treatment, and observing special considerations for patients with African ancestry, children and adolescents, and PLWH (Seedat et al., 2014). The implementation of these guidelines in South Africa has not been systematically examined. Furthermore, the effectiveness of the guidelines in achieving the region-wide goals of blood pressure control in hypertensive patients is unknown. Such assessments are crucial to ensure that the approaches taken to reduce the burden of HT are best suited to the target population. These data are also important for identifying possible factors impeding the successful management of HT in SSA.

Here, we conducted a prospective observational study of newly diagnosed hypertensives from Soweto, South Africa, to assess the current practices and outcomes in health clinics in urban Soweto and built a prospective hypertension cohort for research on factors that may affect response to treatment. Our aims were to capture the characteristics of patients newly diagnosed with hypertension and started on treatment, measure the patient outcomes in terms of those who responded or did not respond to their treatment, and to identify potential predictors of the outcomes, specifically change in BP over time.

2.4 Methods

2.4.1 Study design

The study was a prospective observational study in primary community health centres (CHC) in Soweto, Johannesburg, South Africa. The study sites used included Chiawelo CHC, Itireleng CHC, and several others, including Michael Maponya Provincial CHC, Mofolo South CHC, Orlando Provincial CHC, Zola CHC and Lillian Ngoyi CHC, referred to as *Other Clinics* due to the small enrolment numbers. The University of Witwatersrand's Human Research Ethics Committee (Medical) provided ethics approval (M201143). The approval required sharing a detailed information sheet with participants and obtaining written informed consent before recruitment.

Treatment-naive Black African patients with newly diagnosed hypertension (SBP >140 and/or DBP > 90) were enrolled from August 2021 to March 2023. Participants had to meet all inclusion criteria: 18 years or older, diagnosed with hypertension (SBP > 140 and/or DBP > 90), had not taken anti-hypertensive medication in the past 3 months and not currently taking medication known to affect hypertension. Participants who did not self-identify as black Africans, had a previous diagnosis of TB (within the past 12 months) or being treated for TB, due to leave the Soweto region within 6 months and/or became pregnant during the study, were excluded.

Some of the participants initially visited the health clinics for non-HT related reasons and were recruited into the study when found to meet the inclusion criteria above. Trained nurses used a questionnaire to collect information on demographics (age, self-reported ethnicity), health (history of diabetes, infection, current medications used in the past two months), all newly prescribed medication(s) and doses for HT treatment, and family health history (specifically heart disease), and the participants were measured for height (cm), weight (kg), and waist and hip circumference (cm).

Data for the study were captured using a custom designed REDCap electronic data capture tool (Harris et al., 2009, 2019).

Study visits

Enrolled participants entered the study and provided baseline data; they were requested by the clinic staff to return 7-14 days later for a follow-up visit to monitor their blood pressure. Participants continued to return for follow-up visits until they met the criteria for study exit (controlled SBP and DBP).

Baseline visit

At baseline, study nurses recorded anthropometric (height, weight, and waist and hip circumference) and blood pressure (in triplicate) measurements for each study participant. A blood sample for DNA extraction for future studies was taken from each participant during this visit. The study nurse requested participants to return to the study recruitment office during their follow-up visit to the clinic. To encourage compliance, the research nurse contacted the participants via telephone as a reminder of their follow-up visit, within 7-14 days of their baseline visit. Despite this measure, many participants returned for their follow-up visit much later than the requested date.

Follow-up visit

At each follow-up visit, the study nurse re-recorded anthropometric and blood pressure (in triplicate) measurements for each participant. Any treatment changes were recorded. Participants whose average blood pressure was not within the normal range were invited to donate a blood sample for therapeutic drug monitoring (TDM) analysis. The blood samples were immediately centrifuged and prepared for downstream processing. For those participants whose blood pressure remained elevated, a further follow-up visit was arranged by the study nurse, following the same steps as outlined above.

2.4.2 Definition of phenotypes

BP was measured three times for each participant at each visit using the Omron M6 (Omron Healthcare, Kyoto, Japan) sphygmomanometer. The first measurement was discarded to account for the white coat effect (Pickering et al., 2002). The average of the second and third measurements were used as the blood pressure reading for that visit. Blood pressure control was defined as SBP < 140 and DBP <90 mmHg according to SAHS guidelines (Seedat, 2014). Body mass index (BMI) was measured as weight (kg) divided by the squared height (m^2), as per WHO guidelines.

2.4.3 Statistical analysis

In this work we only used baseline, and first follow-up visit data (no data from additional follow-up visits are reported here). Descriptive statistics included numbers and percentages for categorical variables and mean and standard deviations for continuous variables. Kruskal-Wallis ranked tests were conducted to detect differences in the medians of continuous variables for participants in different treatment sub-groups. The Fisher's exact test was conducted to detect associations between categorical variables of participants in different treatment sub-groups. Median values taken from a previous study conducted on a similar

cohort (Goedecke et al., 2022) were used to address missing waist-circumference data. The participants with missing standing height and/or weight (required for calculating BMI) were excluded from statistical analyses (n=53).

Study participants were grouped by treatment strategy (number and type of drugs). Fisher's exact and Kruskal-Wallis tests were used to identify significant differences in baseline characteristics between groups based on the number of prescribed drugs. Paired-sample *t*-tests were used to quantify the changes in SBP and DBP within the groups. We used multi-level linear regression for longitudinal analysis (Hoffman, 2015) to model the change in BP with a random intercept for each person and fixed effects for age, sex, BMI and waist circumference at baseline, days between visits, and treatment strategy. The baseline and follow-up BP were included in the outcome column accounting for Wilder's principle. The variable `days between visits` was centred at 14 because follow-up visits were intended to occur 14 days after the previous visit. This was done so that the effect of the predictor can be interpreted as the change in BP (mmHg) per day a participant was late to the follow up. The model was built in a stepwise manner such that we started with a null model ($BP \sim 1 + (1 + PID)$) and incrementally added predictors testing for statistical significance and monitoring parsimony. Systolic and diastolic BP outcomes were modelled separately. Maximum likelihood and restricted-maximum likelihood estimation were used to compute the model likelihood. Deviance (-2(Log-likelihood)), Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were indices used to determine the best fitting model (Table S2.1). T-tests, with degrees of freedom estimated through the Satterthwaite method, were used to test for significant predictor estimates, respectively.

All data wrangling, visualizations and statistical analysis were conducted in R (v 4.3.2) and RStudio (v 2023.6.0.42) using lme4 (v 1.1.35.1), lmerTest (v 3.1.3) and tidyverse (v 2.0.0) packages.

2.5 Results

2.5.1 Study participants

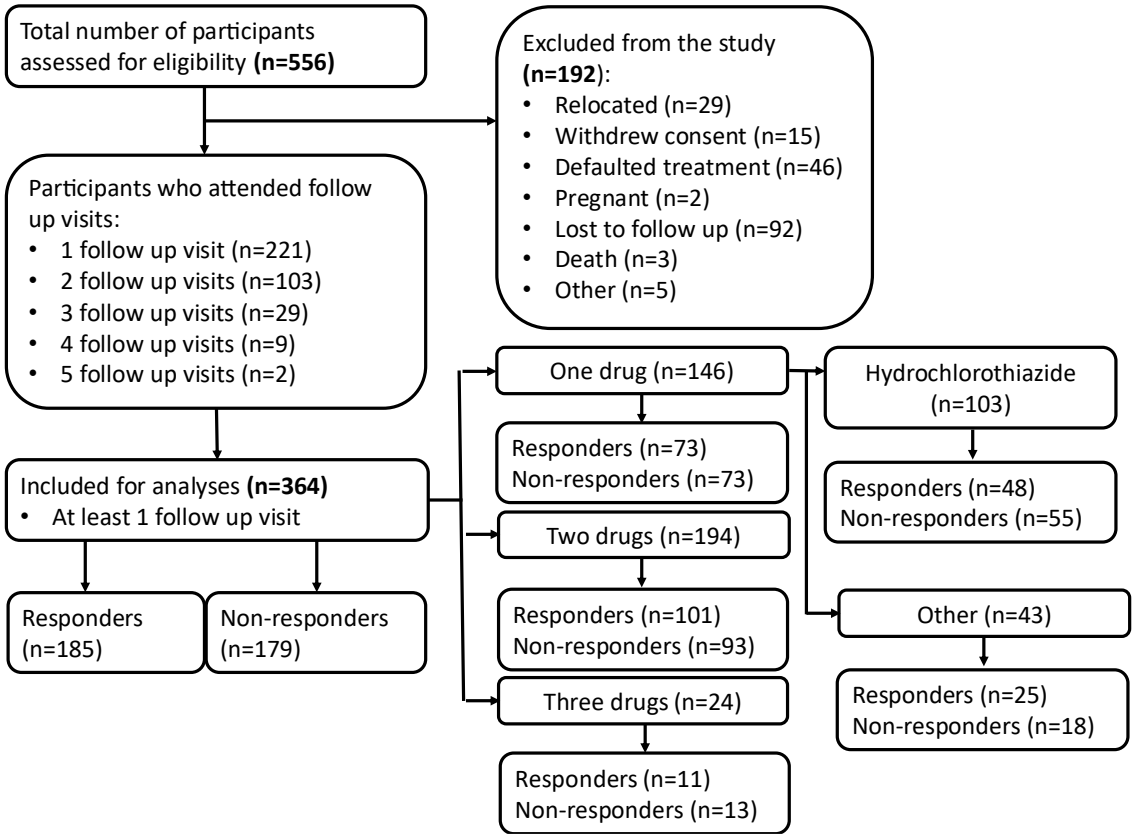


Figure 2. 1: Flow of participants recruited in the study and stratified according to number of drugs prescribed and treatment outcomes.

From the total 556 participants recruited into the study, 364 (65%) satisfied the eligibility criterion of at least one follow-up visit (Figure 1). 'Lost to follow up' (i.e. could not be reached by telephone or follow up date after study close date) was the biggest contributor to participants being excluded from the study.

One or two drugs were prescribed for 93.4% of the participants (Table 2.1). Thiazide-diuretic was prescribed to 70.5% of participants on one-drug therapy and 24.6% of participants were given a calcium channel blocker (Table S2.2). Clinic A (45%) and Clinic B (50%) community health centres contributed most participants. At clinic A, 86% of participants who were given one drug were given a diuretic and 6% were given a calcium channel blocker. At clinic B, 62% of participants who were given 1 drug were given a diuretic and 33% were given a calcium channel blocker. For the participants who were placed on two-drug therapy, 77.3% were given a combination of calcium channel blocker and thiazide-diuretic, 12.3% were given a combination of a thiazide-diuretic and ACE inhibitor, and 9.7% were given a combination of a calcium channel blocker and ACE inhibitor (Table S2.2). At Clinic A, more participants were placed on 2 drugs (63%) compared to 1 drug (31%). Contrastingly, at Clinic B, more participants were placed on 1 drug (51%) compared to 2 drugs (43%). At both clinic A and B, there was no significant difference in the average systolic blood pressure readings for

participants given 1, 2 or 3+ drugs (Table S2.3, S2.4 and S2.5). However, participants (given 2 drugs) at clinic B had a significantly higher diastolic blood pressure compared to those at clinic A. There were more female participants than males. Alcohol consumption was more common than smoking amongst the participants. The number of participants with comorbidities in the cohort was low (6%). The average baseline systolic and diastolic blood pressure readings for the participants prescribed 3 or more drugs were higher than those placed on 1 or 2 drugs.

Table 2. 1: Baseline characteristics of patients, stratified by the number of drugs prescribed

Variables		**Overall** (N=364)	**1 Drug** (N=146)	**2 Drugs** (N=194)	**3+ Drugs** (N=24)
Age (years)[1]		49 (11)	48 (13)	50 (11)	47 (10)
Sex[2]	Female	217 (60%)	87 (60%)	121 (62%)	9 (38%)
	Male	147 (40%)	59 (40%)	73 (38%)	15 (62%)
BMI[1]		30 (7)	30 (7)	30 (8)	31 (6)
Lifestyle factors[2]	Active smokers	72 (19%)	28 (19%)	41 (21%)	3 (13%)
	Alcohol consumers	164 (45%)	66 (45%)	84 (43%)	14 (58%)
Comorbidities[2]	Diabetes	14 (4%)	4 (3%)	9 (5%)	1 (4%)
	Stroke	0 (0)	0 (0)	0 (0)	0 (0)
	Tuberculosis	7 (2%)	4 (5%)	3 (2%)	0 (0%)
Blood pressure (mmHg)[1]	Systolic	157 (21)	156 (21)	157 (20)	166 (29)
	Diastolic	97 (14)	95 (12)	96 (14)	106 (19)
Pulse rate (bpm)		79 (14)	78 (14)	79 (14)	84 (14)
Waist circumference (cm)[1]		93 (16)	91 (16)	94 (16)	98 (13)
Study sites[2]	*Clinic A*	162 (45%)	50 (34%)	102 (53%)	10 (42%)
	Clinic B	182 (50%)	92 (63%)	79 (41%)	11 (46%)
	Other Clinics	20 (5%)	4 (3%)	13 (6%)	3 (12%)

[1]Mean (SD); [2] n (%)

2.5.2 Blood pressure outcomes

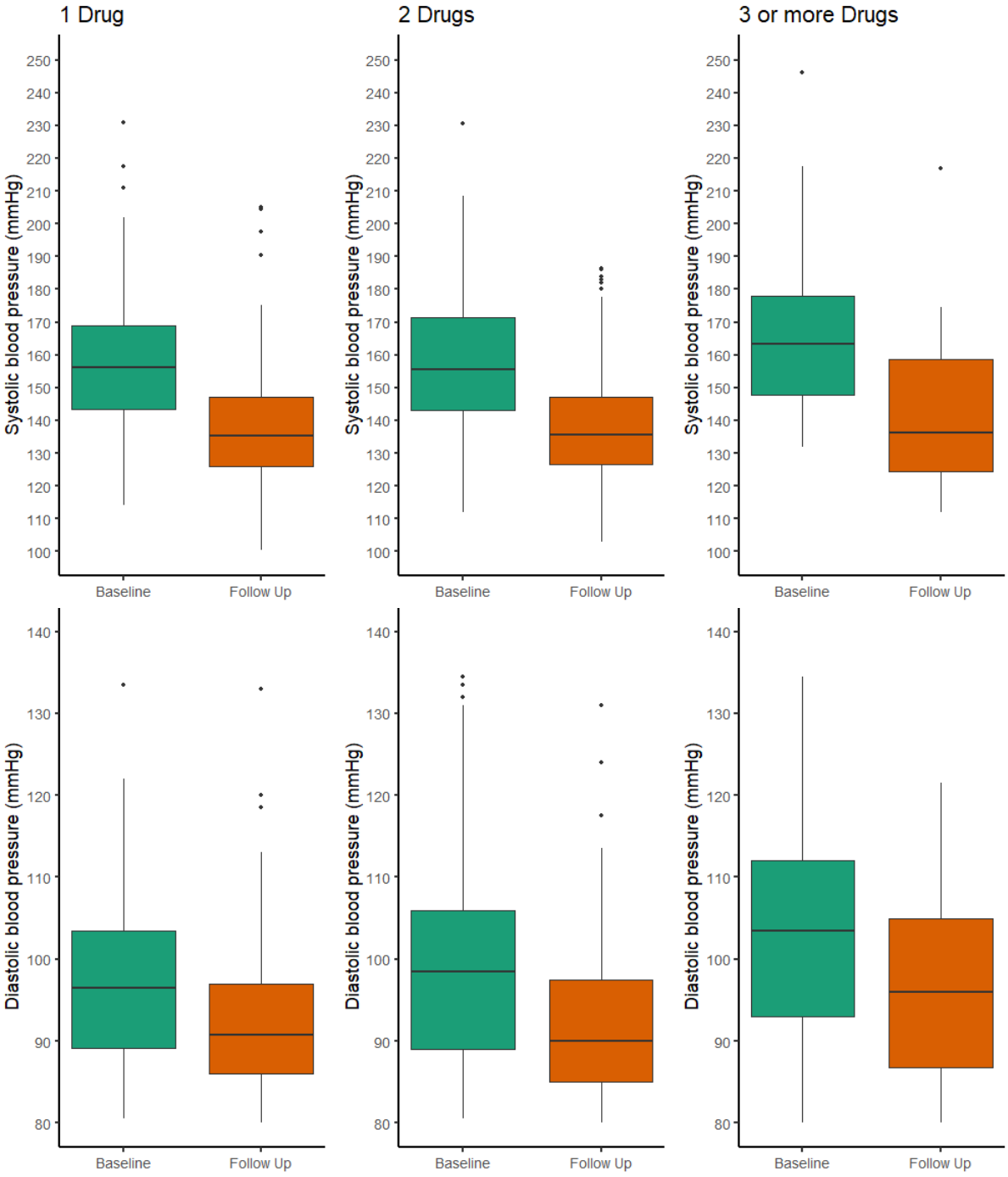


Figure 2. 2: Systolic and diastolic blood pressure (measured in mmHg) readings compared between baseline and first follow up only. The mean time between baseline and first follow up for the participants included in the study was 114 days.

Figure 2.2 shows that there was a reduction in the both the systolic and diastolic blood pressure between baseline and first follow up visit for all participants regardless of the number of drugs they were prescribed. Although the participants were requested (and measures taken to remind and encourage them) to return within 7-14 days, the average time to first follow up was 114 days. The distribution of follow up periods shows that many patients

had their first follow up substantially later than requested (Figure S1). There was no significant difference in the time taken to return for the follow up visit between males and females, within the treatment sub-groups and across the clinics (Table S2.6). The reductions in systolic and diastolic blood pressure between baseline and follow up, for the cohort and in sub-groups according to number of drugs prescribed, were all significant (Table 2.2). The biggest reduction in both systolic and diastolic blood pressure values was in the group of participants who were prescribed 3 or more drugs.

Table 2. 2: Blood pressure values at follow up and reduction in blood pressure change per number of drugs

Systolic Blood Pressure				
Number of drugs(n)	**Baseline BP**	**Follow up BP**	**Reduction in BP (95%CI)**	**p-value**
1 (146)	156.45 ± 21.22	138.50 ± 18.6	17.8 (13.16;22.35)	<0.001
2 (194)	157.16 ± 20.29	138.19 ± 17.9	19 (15.15;22.79)	<0.001
3+ (24)	166.37	144.58 ± 24.60	21.8 (4.69;37.98)	0.013
Diastolic Blood Pressure				
Number of drugs(n)	**Baseline BP**	**Follow up BP**	**Reduction in BP (95%CI)**	**p-value**
1 (146)	95.34 ± 12.4	87.77 ± 11.97	7.57 (5.24;9.89)	<0.001
2 (194)	96.47 ± 14.33	87.47 ± 11.34	8.99 (7.05;10.97)	<0.001
3+ (24)	106.13 ± 18.5	90.62 ± 15.66	15.5 (6.51;24.49)	0.002
Values are mean ± SD in mmHg. BP, blood pressure; SD, standard deviation; paired-sample *t*-test (compared with baseline values)				

Table 2. 3: Blood pressure control rates at first follow up - n (%)

		Overall	Both Uncontrolled n (%)	Systolic BP only n (%)	Diastolic BP only n (%)	Both Controlled n (%)
All patients		364	90 (25)	49 (13)	40 (11)	185 (51)
Sex	Female	217	37 (17)	30 (14)	24 (11)	126 (58)
	Male	147	53 (36)	19 (13)	16 (11)	59 (40)
Number of drugs	1	146	35 (24)	23 (16)	15 (10)	73 (50)
	2	194	45 (23)	24 (12)	24 (12)	101 (52)
	3+	24	10 (42)	2 (8)	1 (4)	11 (46)
Clinics	Clinic A	162	45 (28)	33 (20)	14 (9)	70 (43)
	Clinic B	182	39 (21)	12 (7)	25 (14)	106 (58)
	Other clinics	20	6 (30)	4 (20)	1 (5)	9 (45)

Note: SAHS guidelines for HT control: SBP < 140 mmHg and/or DBP < 90 mmHg for all patients. BP, blood pressure; SAHS, South African Hypertension Society. Values are n (%).

In this study, 51% of participants met the blood pressure control standards, as defined by the guidelines, at first follow up (Table 2.3). There were more females who had controlled blood pressure at first follow up (58%) compared to males (40%). Additionally, there were more males with both uncontrolled systolic and diastolic blood pressure at follow up (36%) compared to females (17%). Blood pressure control was highest among the patients who were prescribed 2 drugs compared to the participants in the other sub-groups, but this was not statistically significant. For the participants on 1 drug, those given a calcium channel blocker had a higher rate of uncontrolled BP compared to those on a diuretic (Table S2.7). A larger percentage of patients given a combination of a calcium channel blocker, and an ACE inhibitor had controlled BP at first follow up compared to patients given a combination of diuretic and ACE inhibitor.

Participants recruited from clinic B had the highest percentage of controlled blood pressure (Table 2.3). Table 2.4 shows the clinical predictors of systolic and diastolic blood pressure values at follow up. The best fitting model was:

$$BP \sim \text{Age} + \text{Sex} + \text{BMI} + \text{Waist Circumference} + \text{Number of drugs} + \text{Clinic} + (1|\text{PID})$$

Table 2. 4: Determinants of blood pressure values at follow up

	Systolic BP		Diastolic BP	
	Estimate	p-value	Estimate	p-value
Intercept (mmHg)	144.8 ± 6.82	<0.001 ***	104.31 ± 4.59	<0.001***
Age (years)	-0.003 ± 0.07	0.959	-0.29 ± 0.048	<0.001***
Sex (female)				
Male	5.092 ± 1.980	0.006**	4.82 ± 1.219	<0.001***
BMI	0.012 ± 0.172	0.940	-0.14 ± 0.106	0.191
WC (cm)	0.064 ± 0.076	0.428	0.100 ± 0.050	0.047*
No. drugs (1 Drug)				
2 Drugs	-0.678 ± 1.84	0.714	0.08 ± 1.139	0.937
3+ Drugs	5.237 ± 3.67	0.154	3.64 ± 2.58	0.107
Clinics (Clinic A)				
Clinic B	-0.189 ± 1.94	0.922	-2.16 ± 1.200	0.073
Other Clinics	9.219 ± 4.430	0.038*	7.95 ± 2.729	0.003**

BP: blood pressure, Values are adjusted slope coefficients ± standard errors in mmHg, Baseline comparison in brackets, WC: waist circumference at baseline. *** - p-value < 0.001, ** - p-value < 0.01, * - p-value < 0.05.

Increasing age was associated with a lower diastolic blood pressure at follow up. Males had significantly higher systolic and diastolic blood pressure values at follow up (compared to females). The number of drugs prescribed to a patient did not affect the level of reduction in blood pressure at follow up. Receiving treatment outside of the two main community health centres was significantly associated with higher systolic and diastolic blood pressure values at follow up, but this group had a small number of participants (n=20).

2.6 Discussion

In this observational prospective study, we explored the characteristics of newly diagnosed hypertensive patients in urban Soweto, South Africa, and reported their treatment outcomes. We observed significant reduction in blood pressure at the first follow-up visit and blood pressure control in about half of the patients. We highlighted differences in treatment approaches across clinics and correlated this with HT outcomes.

Internationally recognized guidelines for treating hypertension have identified a target constant BP of below 140/90 (SBP/DBP) as being ideal for lowering risk of cardiovascular disease (James et al., 2014; Seedat et al., 2014; Unger et al., 2020; Chakraborty et al., 2021; WHO, 2021b). The eighth report of the Joint National Committee for the Prevention, Detection, Evaluation and Treatment of Hypertension (JNC8) (James et al., 2014) recommends combination therapy (2 drugs) for patients with SBP > 20 mmHg and DBP > 10 mmHg above the target BP. In line with these recommendations, the South African Hypertension Society (SAHS) national treatment guideline (Seedat et al., 2014) recommends monotherapy for BP levels up to 19 mmHg and 9 mmHg above the systolic and diastolic BP target respectively, and combination therapy for BP levels beyond that. In this study, 53% of newly diagnosed hypertensives were treated with a two-drug combination and 40% were treated with one drug. Contrary to expectation, there was no difference in baseline SBP and DBP between the two groups: two-drugs, average baseline BP ~157/96 (17 mmHg and 6 mmHg above the target); one drug, had average baseline BP ~156/95 (16 mmHg and 5 mmHg above target). There were clinic-specific differences in treatment approaches with Clinic A prescribing two drugs to 63% (102/162) of participants compared to 43% (79/182) in Clinic B, and one drug to 31% (50/162) of participants compared to 51% (92/182) in Clinic B. This could not be attributed to factors described in the treatment guidelines, including baseline SBP and DBP or co-morbidities, as these were not different between the clinics (Table S2.8 and S2.9). In the Soweto public health care setting, smaller clinics often refer complicated clinical cases to bigger clinics with more capacity. Because clinic A and B are the larger clinics in the area, we expected that their approaches to treating complicated cases (referred from smaller clinics) would be similar. However, the data shows nuanced differences in treatment strategies at these clinics.

Several hypertension treatment guidelines (James et al., 2014; Seedat et al., 2014; Unger et al., 2020; Chakraborty et al., 2021; WHO, 2021b) recommend using diuretics and calcium channel blockers for better outcomes for people who are African or of African descent. We observed that for patients placed on single drug treatment, diuretics were predominantly prescribed (71%) compared to calcium channel blockers (25%). Interestingly at clinic level, although this trend is still present, the proportion of patients who received a calcium channel

blocker at clinic A (6%) was considerably lower than at clinic B (33%). This study highlights that despite similar baseline blood pressure readings, treatment approaches diverge, suggesting nuanced factors influencing clinical decisions at the primary healthcare level. Nonetheless, there's a consistent trend towards utilizing diuretics most often, followed by calcium channel blockers, especially among individuals of African descent.

We observed a significant reduction in blood pressure, from baseline to first follow-up for the cohort overall, regardless of number of drugs prescribed. Hypertension is a complex disease with a complicated aetiology, and therefore simultaneously targeting multiple physiological hypertension pathways (through combination therapy) could result in a greater reduction in blood pressure. However, our data shows that there was no significant difference in BP reduction in patients on two-drug therapy compared to one-drug therapy (Table 2.2). In cases where a single drug was prescribed, 71% received the diuretic and of those 47% controlled their BP at first follow-up; and 25% received a CCB and 55% controlled their BP at first follow-up. When a diuretic and CCB were prescribed as combination therapy (n = 150), 50% controlled their BP at first follow-up. Although, combination therapy is viewed as an important approach for successful hypertension management, in our cohort monotherapy was similarly successful. Reducing the number of antihypertension drugs prescribed has potential implications on the national cost of treating hypertension and may also affect adherence. HCTZ, a thiazide diuretic, has previously been reported to be the cheapest antihypertensive drug in SSA (Twagirumukiza et al., 2010). Further investigation should assess whether monotherapy with a diuretic (or calcium channel blocker, perhaps depending on availability) would be as effective as combination therapy for patients with BP under 160/100 (SBP/DBP) and no exacerbating additional morbidities.

Adherence is a critical factor in the outcomes of hypertension treatment, but we were not able to perform pharmacokinetic analyses to assess this in non-responders. All participants who reported non-adherence at follow-up were excluded from the study. Poor adherence to treatment has been attributed to suboptimal health education (Algabbani and Algabbani, 2020) and low socio-economic status (Schneider et al., 2009), emphasizing the complex interplay between socio-economic factors and adherence in South Africa. Targeted interventions and comprehensive healthcare strategies addressing socio-economic challenges could contribute to achieving better hypertension control through improved adherence.

The clinics in Soweto achieved an excellent 51% BP control (58% in women and 40% in men) at the first return visit compared the published global and SSA figures. A recent study (Zhou et al., 2021) reported that 23% of women globally control their hypertension when on treatment, but only 13% of women in SSA. Similarly, in men, the global and SSA control rates were 18% and 9%, respectively (Zhou et al., 2021). In the AWI-Gen study (Gómez-Olivé et al., 2017), of all participants who were found to be hypertensive, only 18% had controlled blood pressure, and rates of control were higher for women (22%) compared to men (12%). There are notable differences in study design and whereas Zhou *et al. (*2021) was a pooled analysis of data from many different sources the Gómez-Olivé *et al.* (2017) study was a prospective population cross-sectional study of midlife adults in four SSA countries. We do not know how

the control rates achieved in this study compare to the regional, provincial or national control rates as such data are not available.

The effect of treatment duration on outcome requires further investigation. Whereas we expected a follow up period of 7-14 days, the average was 114 days with a wide distribution. In some cases, this may have been due to the restrictions on travel and accessing health care during the COVID-19 pandemic. Another contributing factor could be that once given treatment after the baseline visit, patients are referred back to their initial smaller clinics, and therefore had a delayed follow up visit to the bigger clinic or were lost to follow up. It was both a limitation (because of the introduction of inconsistency in the follow-up period amongst the participants) and strength of the study (because it is a true reflection of the difficulty of follow-up care in treating hypertension in the Soweto setting). Additionally, the "randomness" of the treatment periods in the participants increases our confidence that the control rates were not significantly influenced by a treatment period effect. Analyses also showed that there was no significant difference in treatment/follow up periods between men and women, across participants in the different treatment sub-groups and across the different clinics. The variable denoting treatment/follow up period also did not significantly improve the predictive mixed model for this cohort. According to the mixed model, men were likely to have higher SBP and DBP readings at first follow up, compared to women. Differential response between men and women has frequently been reported, for example in the CREOLE study, African women on two-drug therapy controlled their blood pressure better than men (Ojji et al., 2019). In the US, men with African ancestry have lower rates of control and higher rates of hypertension-related mortality compared to women (Bulgin et al., 2021). The factors contributing to this sex difference are not well understood. It has been hypothesized that stress management (Fonkoue et al., 2018) and sex-specific RAAS sensitivity (Leete and Layton, 2019) contribute to differential responses in men and women with African ancestry. Further research is needed to elucidate these factors and develop tailored interventions to improve hypertension management, particularly among men with African ancestry.

Although the mechanisms driving hypertension treatment response and outcomes are poorly understood, there is evidence that demographic, behavioural and genetic factors play a role. Genetic variants in genes influencing the pharmacodynamics of drug response have been previously implicated (e.g. *ADD1, GNB3, ADRB1, NOS3, ACE, AGTR1, CYP11B2*) (Schwartz and Turner, 2004). Some of the single nucleotide polymorphisms associated with drug response were found to be present at different frequencies among South African ethnic groups (Masilela et al., 2020). Furthermore, rates of potassium and aldosterone excretion (Ripley et al., 2000), and creatine kinase-mediated blocking of sodium retention (Brewster et al., 2006), are higher in people of African origin, suggesting an anticipated higher response rate to thiazide therapy for hypertension. We hypothesize that there are additional genetic variants which are associated with treatment response in South African hypertensives, which need to be investigated in future studies. A similar study with a larger sample size and more follow up points could generate additional insights into the associations between demographic characteristics, behaviours, genetic variations, and physiological markers, with patterns and trends of treatment response across different populations thereby playing an important role

in informing clinical practice and guiding future research efforts in hypertension management.

2.7 Perspectives

In this observational prospective study in urban Soweto, South Africa, the characteristics and treatment outcomes of newly diagnosed hypertensive patients were investigated. Significant reductions in blood pressure were observed at the first follow-up visit, with approximately half of the patients achieving blood pressure control. The treatments diverged from the national recommendations for hypertension treatment, with 53% of patients receiving a two-drug combination therapy at initial diagnosis. Monotherapy was most often with a diuretic, but calcium channel blockers were frequently prescribed. Notably, patients on monotherapy achieved similar reductions (compared to participants on two-drug therapy) in blood pressure at first follow up, suggesting that it is effective in an urban South African setting. Soweto clinics demonstrated higher control rates compared to previous reports from SSA, particularly in women. However, there remain challenges to optimal treatment and control including adherence to medication regimens and inconsistent patient follow-up, necessitating targeted interventions taking into consideration socio-economic and health system factors. Additionally, evidence of genetic variants previously found to influence drug response in European and American populations needs to be assessed among different South African ethnic groups (Masilela et al., 2020) and could lead to the discovery of pharmacogenomic differences in hypertension treatment responses. Overall, this study underscores the potential of observational studies to provide valuable insights into understanding factors influencing hypertension treatment effectiveness, complementing controlled trials by reflecting clinical practice.

2.8 Acknowledgements

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2.9 Supplementary Materials

Table S2. 1: Assessment of mixed model fit by comparing AIC, BIC and deviance.

Model	AIC	BIC	-2LL
Systolic BP			
1 + (1|PID)	6577.535	6591.306	6571.535
Age + (1|PID)	6582.807	6601.169	6574.807
Age + Sex + (1|PID)	6573.321	6596.272	6563.321
Age + Sex + BMI + (1|PID)	6110.546	6137.634	6098.546
Age + Sex + BMI + WaistCircum + (1|PID)	6114.399	6146.002	6100.399
Age + Sex + BMI + WaistCircum + Number of drugs + (1|PID)	6103.605	6148.752	6083.605
Age + Sex + BMI + WaistCircum + Number of drugs + DaysBetweenVisits + (1|PID)	6113.294	6162.956	6091.294
Age + Sex + BMI + WaistCircum + Number of drugs + DaysBetweenVisits + Clinic + (1|PID)	5971.811	6043.76	5939.811
Age + Sex + BMI + WaistCircum + Number of drugs + Clinic + (1|PID)	5962.35	6029.802	5932.35
Diastolic BP			
Age + (1|PID)	5852.023	5870.384	5844.023
Age + Sex + (1|PID)	5828.337	5851.289	5818.337
Age + Sex + BMI + (1|PID)	5427.731	5454.819	5415.731
Age + Sex + BMI + WaistCircum + (1|PID)	5423.851	5455.545	5409.851
Age + Sex + BMI + WaistCircum + Number of drugs + (1|PID)	5415.305	5460.452	5395.305
Age + Sex + BMI + WaistCircum + Number of drugs + DaysBetweenVisits + (1|PID)	5425.808	5475.47	5403.808
Age + Sex + BMI + WaistCircum + Number of drugs + DaysBetweenVisits + Clinic + (1|PID)	5291.944	5363.892	5259.944
Age + Sex + BMI + WaistCircum + Number of drugs + Clinic + (1|PID)	5281.918	5349.37	5251.918

Table S2. 2: Distribution of drug classes and combinations within different treatment sub-groups. Participants were placed into sub-groups depending on how many drugs they were prescribed.

Drug class	Number of participants, n(%)
1-Drug Therapy (N = 146)	
ACEi	4 (2.7)
CCB	36 (25)
Diuretic	103 (71)
α-blocker	3 (2.1)
2-Drug therapy (N=194)	
ACEixα-blocker	1 (1)
CCBxACEi	19 (10)
DiureticxACEi	24 (12)
DiureticxCCB	150 (77)
3-Drug therapy (N=22)	
DiureticxACEixCCB	21 (95)
DiureticxCCBxCCB	1 (5)
4-Drug therapy (N=2)	
DiureticxCCBxACEixOther	
DiureticxDiureticxCCBxDiuretic	
ACEi denotes ACE inhibitor, CCB denotes calcium channel blocker, combinations are denoted by drug classes separated by "x"	

Table S2. 3: Comparison of baseline characteristics and drug class distribution for participants (treated with 1 drug) across the two main clinics.

Variable	Clinic A , N = 50[1]	Clinic B N = 92[1]	p-value[2]
drug_class			<0.001
ACEi	2 (4.0%)	2 (2.2%)	
CCB	3 (6.0%)	33 (36%)	
Diuretic	43 (86%)	57 (62%)	
α-blockers	2 (4.0%)	0 (0%)	
Age	49 (13)	48 (12)	0.7
Sex			0.10
Female	25 (50%)	59 (64%)	
Male	25 (50%)	33 (36%)	
BMI	30 (9)	30 (6)	0.6
Active smokers	9 (18%)	19 (21%)	0.7
Alcohol consumers	25 (50%)	41 (45%)	0.5
Diabetes	1 (2.0%)	3 (3.3%)	>0.9
Hypertension	4 (8.0%)	21 (23%)	0.027
Stroke			
No	50 (100%)	92 (100%)	
Tuberculosis	0 (0%)	4 (4.3%)	0.3
Systolic blood pressure(mmHg)	157 (22)	156 (21)	0.8
Diastolic blood pressure(mmHg)	96 (12)	94 (12)	0.3
Pulse rate(bpm)	79 (15)	78 (14)	>0.9
Waist circumference(cm)	96 (15)	89 (16)	0.025

[1] n (%); Mean (SD)

[2] Fisher's exact test; Wilcoxon rank sum test; Pearson's Chi-squared test

ACEi denotes ACE inhibitor, CCB denotes calcium channel blocker

Table S2. 4: Comparison of baseline characteristics and drug class distribution for participants (treated with 2 drugs) across the different clinics.

Variable	Clinic A , N = 102[1]	Clinic B , N = 79[1]	p-value[2]
drug_class			0.021
ACEixα-blockers	1 (1.0%)	0 (0%)	
CCBxACEi	9 (8.8%)	9 (11%)	
DiureticxACEi	19 (19%)	4 (5.1%)	
DiureticxCCB	73 (72%)	66 (84%)	
Age	50 (11)	50 (11)	0.8
Sex			0.10
Female	59 (58%)	55 (70%)	
Male	43 (42%)	24 (30%)	
BMI	30 (8)	30 (8)	0.8
Active smokers	23 (23%)	16 (20%)	0.7
Alcohol consumers	45 (44%)	32 (41%)	0.6
Diabetes	3 (2.9%)	6 (7.6%)	0.2
Hypertension	3 (2.9%)	21 (27%)	<0.001
Stroke			
No	102 (100%)	79 (100%)	
Tuberculosis	0 (0%)	2 (2.5%)	0.2
Systolic blood pressure(mmHg)	158 (22)	155 (18)	0.5
Diastolic blood pressure(mmHg)	98 (15)	93 (12)	0.008
Pulse rate(bpm)	81 (14)	75 (14)	0.015
Waist circumference(cm)	97 (14)	88 (18)	<0.001

[1] n (%); Mean (SD)

[2] Fisher's exact test; Wilcoxon rank sum test; Pearson's Chi-squared test

ACEi denotes ACE inhibitor, CCB denotes calcium channel blocker, combinations are denoted by drug classes separated by "x".

Table S2. 5: Comparison of baseline characteristics and drug class distribution for participants (treated with 3 or more drugs) across the different clinics.

Variable	Clinic A N = 10[1]	Clinic B N = 11[1]	p-value[2]
drug_class			>0.9
DiureticxACEixCCB	9 (90%)	10 (91%)	
DiureticxCCBxCCB	1 (10%)	0 (0%)	
DiureticxDiureticxCCBxDiuretic	0 (0%)	1 (9.1%)	
Age	50 (9)	44 (10)	0.2
Sex			0.4
Female	3 (30%)	6 (55%)	
Male	7 (70%)	5 (45%)	
BMI	31.7 (5.4)	31.3 (7.7)	>0.9
Active smokers	0 (0%)	3 (27%)	0.2
Alcohol consumers	5 (50%)	8 (73%)	0.4
Diabetes	0 (0%)	1 (9.1%)	>0.9
Hypertension	1 (10%)	2 (18%)	>0.9
Stroke			
No	10 (100%)	11 (100%)	
Tuberculosis			
No	10 (100%)	11 (100%)	
Systolic blood pressure(mmHg)	150 (15)	169 (25)	0.062
Diastolic blood pressure(mmHg)	97 (13)	107 (14)	0.14
Pulse rate(bpm)	84 (12)	87 (14)	0.9
Waist circumference(cm)	101 (9)	97 (15)	0.5

[1] n (%); Mean (SD)

[2] Fisher's exact test; Wilcoxon rank sum test; Wilcoxon rank sum exact test

ACEi denotes ACE inhibitor, CCB denotes calcium channel blocker, combinations are denoted by drug classes separated by "x".

Table S2. 6: Follow up periods (in days) compared between sex, number of drugs at baseline and clinic. Values are mean (SD).

Sex	**Male**		**Female**	**p-value**
	108.52		**117.97**	**0.4311**
Number of drugs at baseline	**1 drug**	**2 drugs**	**3+ drugs**	
	108.76(116.8)	**119.26(111.76)**	**105.66(104.29)**	**0.174**
Clinic	**Clinic A**	**Clinic B**	**Other Clinics**	
	119.56(120.12)	**108.82(104.4)**	**118.85(133.88)**	**0.763**

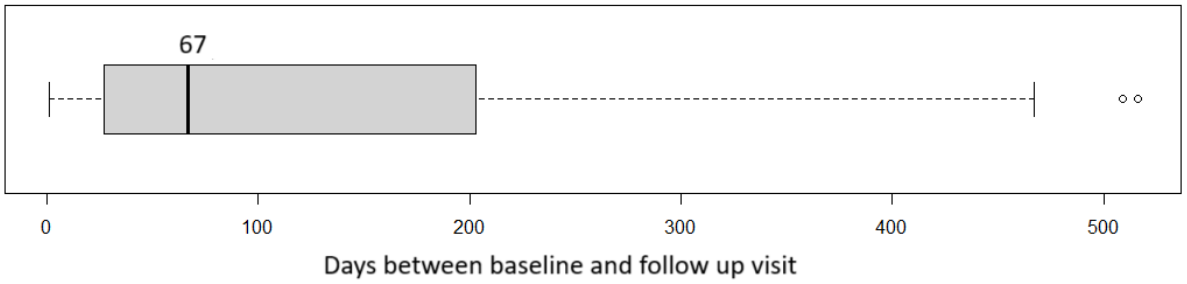


Figure S2. 1: Boxplot showing the distribution of follow up periods between the baseline visit and follow up. Median = 67 days and mean = 114.16 days

Table S2. 7: Blood pressure control rates by drug class for participants on different number of drugs, at first follow up - n (%)

	Both Uncontrolled	Systolic BP only	Diastolic BP only	Both Controlled
1 Drug				
Overall (146)	35 (24)	23 (16)	15 (10)	73 (50)
Diuretic (103)	24 (23)	20 (19)	11 (11)	48 (47)
Calcium Channel Blocker (36)	11 (31)	1(3)	4(11)	20(55)
ACE inhibitor (4)	0	0	0	4 (100)
α-blocker (3)	0	2 (67)	0	1(33)
2 Drugs				
Overall (194)	45 (24)	24 (12)	24 (12)	101 (52)
Diuretic + Calcium Channel Blocker (150)	37 (24)	19 (13)	19 (13)	75 (50)
Diuretic + ACE inhibitor (24)	5 (20)	4 (17)	3 (13)	12 (50)
Calcium Channel Blocker + ACE inhibitor (19)	2 (11)	1 (5)	2 (11)	14 (73)
ACE inhibitor + α-blocker (1)	1 (100)	0	0	0
3 or more Drugs				
Overall (24)	10 (42)	2 (8)	1 (4)	11 (46)
Diuretic + Calcium Channel Blocker + ACEi (22)	10 (45)	2 (9)	1 (5)	9 (41)
Diuretic + Calcium Channel Blocker (2x) (1)	0	0	0	1 (100)
Diuretic (3x) + Calcium Channel Blocker (1)	0	0	0	1 (100)

Note: SAHS guidelines for HT control: SBP < 140 mmHg and/or DBP < 90 mmHg for all patients. BP, blood pressure; SAHS, South African Hypertension Society. Values are n (%)

Table S2. 8: Baseline characteristics for all participants recruited at Clinic A.

Variable	1-Drug therapy, N = 50[1]	2-Drug therapy, N = 102[1]	3-Drug therapy, N = 10[1]
Age(years)	49 (13)	50 (11)	50 (9)
Sex			
Female	25 (50%)	59 (58%)	3 (30%)
Male	25 (50%)	43 (42%)	7 (70%)
BMI	30 (9)	30 (8)	32 (5)
Active smokers	9 (18%)	23 (23%)	0 (0%)
Alcohol consumers	25 (50%)	45 (44%)	5 (50%)
Diabetes	1 (2.0%)	3 (2.9%)	0 (0%)
Stroke			
No	50 (100%)	102 (100%)	10 (100%)
Tuberculosis			
No	50 (100%)	102 (100%)	10 (100%)
Systolic blood pressure(mmHg)	157 (22)	158 (22)	150 (15)
Diastolic blood pressure(mmHg)	96 (12)	98 (15)	97 (13)
Pulse rate(bpm)	79 (15)	81 (14)	84 (12)
Waist circumference(cm)	96 (15)	97 (14)	101 (9)

[1] Mean (SD); n (%)

Table S2. 9: Baseline characteristics for all participants recruited from clinic B.

Variable	1-Drug therapy, N = 92[1]	2-Drug therapy, N = 79[1]	3-Drug therapy, N = 10[1]	4-Drug therapy, N = 1[1]
Age(years)	48 (12)	50 (11)	41 (7)	68 (NA)
Sex				
Female	59 (64%)	55 (70%)	6 (60%)	0 (0%)
Male	33 (36%)	24 (30%)	4 (40%)	1 (100%)
BMI	30 (6)	30 (8)	31 (8)	39 (NA)
Active smokers	19 (21%)	16 (20%)	3 (30%)	0 (0%)
Alcohol consumers	41 (45%)	32 (41%)	7 (70%)	1 (100%)
Diabetes	3 (3.3%)	6 (7.6%)	1 (10%)	0 (0%)
Stroke				
No	92 (100%)	79 (100%)	10 (100%)	1 (100%)
Tuberculosis	4 (4.3%)	2 (2.5%)	0 (0%)	0 (0%)
Systolic blood pressure(mmHg)	156 (21)	155 (18)	170 (26)	154 (NA)
Diastolic blood pressure(mmHg)	94 (12)	93 (12)	107 (14)	105 (NA)
Pulse rate(bpm)	78 (14)	75 (14)	87 (15)	96 (NA)
Waist circumference(cm)	89 (16)	88 (18)	95 (14)	121 (NA)

[1] Mean (SD); n (%)

Chapter 3: Genetic Variation in Hydrochlorothiazide-Response Related Genes among Hypertensive Individuals in Soweto, South Africa

This chapter contains the contents of a manuscript set out to achieve objectives 2 and 3: to describe the genetic variation in the HCTZ response-related genes in newly diagnosed hypertensives treated at primary health centres in Soweto, South Africa. Clinical trials have shown that there is differential response to antihypertension medication across different populations. Genetic analyses stemming from these clinical trials have identified multiple genetic loci associated with response to antihypertension medication. Understanding the relationship between population-specific genetic variation and treatment response has the potential to inform treatment guidelines to improve treatment effectiveness and minimize adverse effects. This chapter demonstrates that there may be unique genetic variation in continental Africans which potentially contributes to the response to HCTZ. This suggests that there is a need to invest in more genetic studies in underrepresented populations to better understand the genetics of hypertension and hypertension treatment response.

The work described in this chapter has been submitted for publication to *Clinical and Translational Science*.

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KN, MR and CM designed the study. KN and ML recruited the participants. KN generated the sequencing data. KN and DT conducted the genetic analyses. KN, DT and AM wrote the manuscript. MR, AM and CM edited the manuscript, and the manuscript was reviewed and approved by all authors.

3.2 Abstract

Africa bears the largest burden of morbidity and mortality for hypertension (HT) and cardiovascular diseases. Thiazide diuretics, including hydrochlorothiazide (HCTZ), are the most prescribed hypertension medicines globally due to their cost effectiveness and minimal side effect (as part of combination therapy). Response to HCTZ is affected by genetic variation, among other factors. However, the impact of genetics on HCTZ response in Africans is unknown. This study examines genetic variation in HCTZ response-related genes among newly diagnosed hypertensives in Soweto, South Africa. Using targeted next-generation sequencing of 16 genes in 364 HT patients who had at least one follow-up visit, we identified 1258 variants in key HCTZ response genes. Among these, 89 were novel variants, of which 12 are protein-altering and 8/12 are predicted to be deleterious. We found population-specific allele frequency differences for variants previously associated with HCTZ response. Although we did not replicate prior associations (e.g., rs2611316 in *ALDH1A2*) in non-African populations, carriers of the intronic variant rs1025450259 in *GNB3* had significantly greater reduction in blood pressure compared to other patients on HCTZ. This research underscores the importance of pharmacogenetic studies in the African context. Given the limitations of our study and existing *in silico* prediction tools, larger, controlled trials are needed to assess genetic influences on HT treatment outcomes, advancing personalized medicine for African populations.

3.3 Introduction

Cardiovascular diseases (CVDs) are among the leading causes of morbidity and mortality globally (WHO, 2021a). In the sub-Saharan Africa (SSA) region, the burden of CVDs is increasing. In 2021, CVDs were reported as the second leading cause of all deaths in SSA (11%) (GBD Compare Data Visualization, 2021), with hypertension (HT) the leading risk factor (Seeley et al., 2020). In South Africa, the burden of HT is alarmingly high and contributes substantially to cardiovascular disease mortality (Gómez-Olivé et al., 2017; Abdelatif et al., 2021). Improving HT management and treatment outcomes, therefore, remains a public health priority for South Africa and the African continent.

Several evidence-based treatment guidelines have been published for the management of elevated blood pressure and HT (Seedat et al., 2014; Unger et al., 2020). Blood pressure (BP) control is a critical outcome for HT management directly impacting risk of death and other CVD-related complications (WHO, 2021b). HT management guidelines often contain specific recommendations for individuals with African ancestry (Unger et al., 2020) who have higher rates of low renin salt-sensitive hypertension and adverse cardiac outcomes compared to individuals of European ancestry (Faconti et al., 2020). Furthermore, international clinical trials have shown population-specific differences in BP response to antihypertensive drugs (Johnson, 2008; Lackland, 2014). Diuretics and calcium channel blockers have been found to elicit a better BP response in African Americans compared to European Americans (Johnson, 2008). Similarly, in sub-Saharan African populations, diuretics with calcium channel blocker dual therapy tends to be more effective compared to dual therapy with ACE inhibitors with beta-blockers (Ojji et al., 2019). As a result, HT management guidelines recommend treatment with either diuretics or calcium channel blockers as first line therapy for individuals with African ancestry (Seedat et al., 2014; Unger et al., 2020; WHO, 2021b).

Studies have also shown that diuretics are the most cost effective first-line strategy for treating hypertension in many countries (Park et al., 2020; Kučan et al., 2021). Among these extensively prescribed diuretics, hydrochlorothiazide (HCTZ) is the leading prescribed medication, alone or in combination, in sub-Saharan Africa (Seeley et al., 2020; Cavagna et al., 2023). HCTZ, a thiazide diuretic, inhibits sodium reabsorption in the distal convoluted tubules of the kidney (Herman et al., 2024b) and is particularly beneficial as part of combination therapy as it blunts water/sodium retention effects of other antihypertension drug classes (Skolnik et al., 2000).

According to genome-wide association studies (GWAS) on participants in large clinical trials, the interindividual response to HCTZ and other antihypertensives is influenced by genetics (Rysz et al., 2020). For example, variants in the HMA-CoA synthase gene (*HMGCS)* have been associated with adverse reactions to HCTZ (HCTZ) in African and European Americans (Singh et al., 2018). Furthermore, variation in important BP regulating genes *ACE, ADD1* and *NEDD4L* have been associated with varied responses to HCTZ in multiple populations (Sciarrone et al., 2003; Svensson-Färbom et al., 2011). Understanding the contribution of genetics in HCTZ response is therefore particularly important for diverse biogeographical groups, such as SSA, which rely heavily on the drug for managing hypertension.

The role of genetic variation in antihypertensive drug response in continental African populations is not well understood, as genetic studies in these populations are scarce (Radouani et al., 2020). Addressing this gap is an important step in optimizing treatment efficacy, preventing adverse reactions, and maximising cost-effectiveness in hypertension management in Africa. In this exploratory study, we evaluated the role of genetic variation in drug response by next generation sequencing of 16 known HCTZ response-related candidate genes, in newly diagnosed hypertensives in Soweto, South Africa. We hypothesize that, in addition to known variants, unique genetic variations within African populations contribute to the efficacy of HCTZ, influencing HT management.

3.4 Materials and Methods

3.4.1 Study participants

This study was approved by the University of Witwatersrand, Human Research Ethics Committee (Medical) (reference numbers: M220286 and M201143). Participants were recruited from community health clinics in Soweto, South Africa. Informed consent was obtained from all participants prior to enrolment in the study. Inclusion criteria were adults, 18 years or older who had been diagnosed with hypertension (SBP > 140 and/or DBP > 90) but had not taken anti-hypertensive medication in 3 months, prior to enrolment; were not taking other medications known to affect hypertension prior to enrolment in the study; and non-pregnant. To be included in this study the participants were required to return for at least one study follow-up visit at which BP was measured. At baseline, study nurses recorded anthropometric and blood pressure measurements and obtained a venous blood sample in the anticoagulant, Ethylenediaminetetraacetic acid (EDTA), for genetic analyses. At follow-up visit(s), anthropometric and blood pressure measurements were recorded along with treatment adherence (self-reported) and if there had been any alterations to the original prescription for HT medication at baseline. Blood pressure control, i.e. positive response to

treatment, was determined as SBP<140 and DBP<90. A responder is defined as having controlled BP at their study follow-up.

3.4.2 Sample preparation

Genomic DNA was extracted from blood buffy coat samples using the Chemagic360 (PerkinElmer Inc., Waltham, Massachusetts). DNA quality and quantity were assessed using spectrophotometry (a ratio of absorbance at 280/260 nm of >2 suggested protein contamination and a ratio of absorbance at 230/260 nm of >2 suggested the presence of excess salt and other contaminants). DNA was quantified on the Qubit 3 fluorometer using the Qubit dsDNA broad range assay kit (Invitrogen, Waltham, Massachusetts). The AmpliSeq Library PLUS kit (Illumina Inc, San Diego, California) was used to prepare the sequencing libraries according to manufacturer's instructions. Target regions of candidate genes were amplified using primer pools, as part of the library preparation process.

3.4.3 Candidate gene selection and panel design

Candidate genes, namely *ADD1, RAD52, WNK1, NEDD4L, ACE, AGTR1, YEATS, NOS3, PRKCA, GNB3, ALDH1A2, TXNDC11, TTC6, SCNN1G, HSD31B* and *ADO,* were selected based on a prioritization approach of previous evidence of a role in HCTZ drug response in PharmGKB (Whirl-Carrillo et al., 2021). A custom targeted gene panel was designed using the DesignStudio tool (Illumina Inc, San Diego, California). The primers were designed to target the exons, exon-intron boundaries, 5' and 3' untranslated regions and regulatory regions of the candidate genes aiming for amplicons of approximately 150 bp in length (Supplementary table S3.1). The total size of the panel of 737 amplicons for 16 genes was 103,973bp and the probes covered 99.1% of the target regions.

3.4.4 High-coverage targeted sequencing and variant analysis

The Illumina iSEQ 100 (Illumina Inc, San Diego, California), using 2 x 150bp paired-end chemistry, was used to sequence libraries of pooled amplicons loaded onto a standard flow-cell, according to manufacturer's instructions. Sequencing performance was monitored using a PhiX (1%) positive control. The Illumina sequencing analysis viewer was used to assess sequencing quality. Raw sequencing reads were mapped to the human genome build GRCh38/hg38 using Burrow-Wheeler Aligner (BWA-MEM) (Li and Durbin, 2009). Off target intergenic variants were excluded. The Genome Analysis Toolkit (GATK) tools including: HaplotypeCaller, GenomicsDBImport, GenotypeGVCFs and SelectVariants (McKenna et al., 2010) were applied for SNP and INDEL joint-calling for all samples (DePristo et al., 2011; Auwera and O'Connor, 2020). The full workflow used, and parameters are available from (https://github.com/Kudashian/PGxHT). For variant filtration, thresholds for quality-by-depth (QD), Fisher strand (FS), Strand Odds Ratio (SOR), mapping quality (MQ), mapping quality rank sum test (MQRankSum) and read position rank sum test (ReadPosRankSum) were used to hard-filter.

3.4.5 Variant annotation and predictions

Variants were annotated and classified using the Ensembl Variant Effect Predictor (VEP) web interface (McLaren et al., 2016). Scores from the Combined Annotation Dependent Depletion (CADD) (Kircher et al., 2014) tool were used to assess the potential protein-altering effect of all missense, splice, frameshift, insertion/deletion and stop gain variants. CADD integrates

multiple annotations into a single metric and therefore provides a more robust prediction. Scores from the Rare Exome Variant Ensemble Learner (REVEL) tool were used as additional evidence to predict the impact of missense variants. REVEL, a meta-predictor, predicts deleteriousness on the basis of 13 other prediction tools (Ioannidis et al., 2016). Similarly, SpliceAI (Jaganathan et al., 2019), SIFT Indel (Hu and Ng, 2013) and LOFTEE (Karczewski et al., 2020) were used as additional evidence for predicting the impact of splice, insertion/deletion and frameshift variants, respectively.

3.4.6 Statistical analysis

Association analyses were performed using Plink v.1.9 (https://www.cog-genomics.org/plink/1.9/) (Purcell et al., 2007). Variants in linkage disequilibrium (LD) with other variants in the cohort, were pruned from the call set (multiple correlation coefficient over 0.5). Logistic regression was used to investigate the association between genetic variants and treatment outcomes. In this analysis, blood pressure control (systolic and diastolic blood pressure equal to or below 140/90, respectively) was used as the outcome with age, sex, BMI and whether HCTZ was part of treatment as covariates. Additionally, we regressed reduction in systolic and diastolic blood pressure with genetic variation, using the same covariates. This was done to investigate variants which may not be associated with BP control but with BP reduction in response to treatment. Because our candidate genes were chosen based on prior knowledge of association to HCTZ treatment outcomes we used linear and logistic regression to investigate whether there was an association between genetic variants and treatment outcomes in individuals treated with HCTZ only (n=103). The genetic models used to analyze this data were genotypic and additive models. The Bonferroni corrected p-value threshold was calculated as 0.05/number of variants tested. All data wrangling and visualizations were done in R (v 4.3.2) and RStudio (v 2023.6.0.42) using the tidyverse (v 2.0.0) and smartsnp (v 1.1.0) packages.

3.5 Results

3.5.1 Study participants

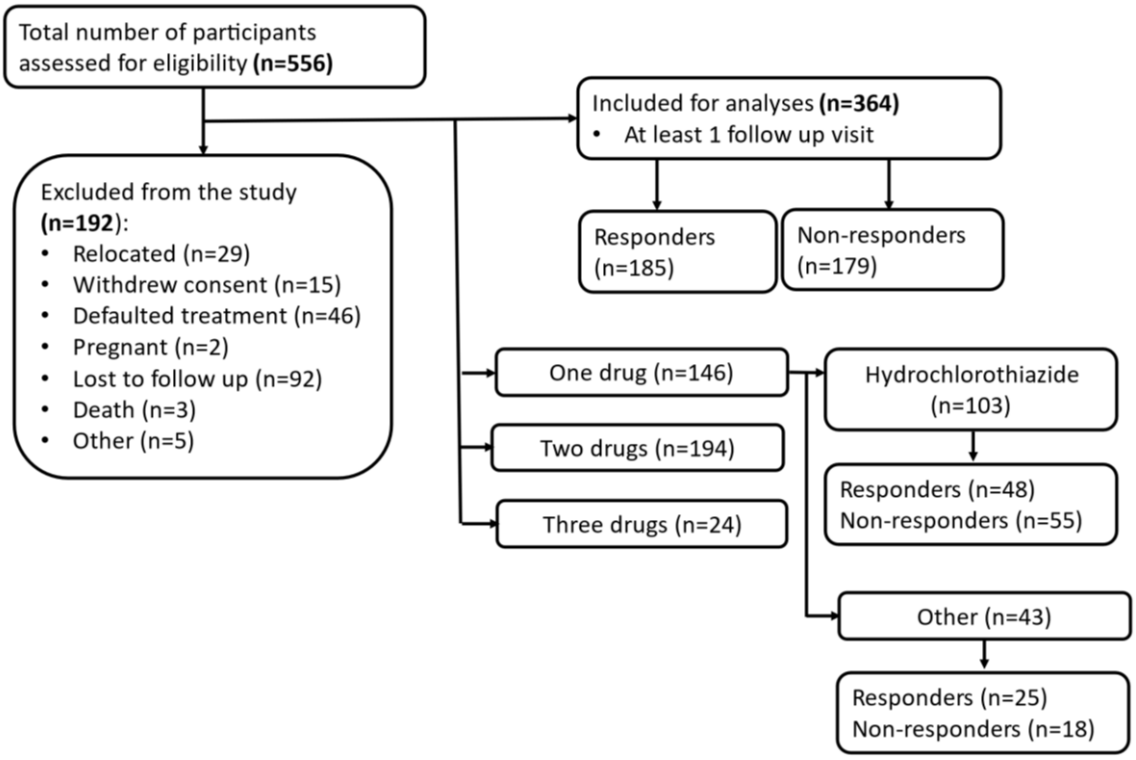


Figure 3. 1: Flow of participants recruited into the study and stratified according to groups of interest: Overall responders and non-responders, and responders and non-responders in individuals treated with HCTZ monotherapy. Responders; participants with BP (SBP/DBP) below 140/90 mmHg.

A total of 556 participants (Fig. 4.1) with hypertension were enrolled in the study and data was collected on the treatment prescribed by the treating clinician. Of these, 364 (65%) returned for at least one follow-up visit and were eligible for this genetic study. About half of the participants in the study (185/364) reached blood pressure control in response to treatment at first follow up and of those 26% were on HCTZ monotherapy. The proportion of responders was higher in females compared to males (Table 3.1). Baseline characteristics including BMI, waist circumference, age and sex, and average systolic and diastolic blood pressure were similar between responders and non-responders (Table 3.1).

Table 3. 1: Baseline characteristics of patients, stratified as responders and non-responders to treatment at first follow up visit

Variables		Overall (N=364)	Responders (N=185)	Non-responders (N=179)
Age (years)[1]		49 (11)	50 (11)	48 (12)

Sex	Female	217 (60%)	126 (68%)	91 (51%)
	Male	147 (40%)	59 (32%)	88 (49%)
BMI[1]		30 (7)	30 (7)	30 (8)
Lifestyle factors	Active smokers	72 (19%)	33 (18%)	39 (22%)
	Alcohol consumers	164 (45%)	74 (40%)	90 (50%)
Comorbidities	Diabetes	14 (4%)	9 (5%)	5 (3%)
	Stroke	0 (0)	0 (0)	0 (0)
	Tuberculosis	7 (2%)	4 (2%)	3 (2%)
Blood pressure (mmHg)[1]	Systolic	157 (21)	154 (21)	161 (21)
	Diastolic	97 (14)	94 (14)	100 (14)
Pulse rate (bpm)		79 (14)	77 (14)	81 (14)
Waist circumference (cm)[1]		93 (16)	93 (16)	93 (16)
Number of drugs at baseline	One Drug	146 (40%)	73 (39%)	73 (41%)
	Two Drugs	194 (53%)	101 (55%)	93 (52%)
	3 or more Drugs	24 (7%)	11 (6%)	13 (7%)

[1]Mean (SD); [2] n (%)

3.5.2 Variant discovery, annotation and prediction

The amplicons from the 364 samples were sequenced over 8 runs and each run had a Q30 value above 90% (for each run, 90% of the reads were sequenced with an error rate of 1/1000). The mean sequencing depth for the panel was 172x (SD=15.77) and the mean sequencing depth for amplicons in each gene was above 100x reads (Supp. Fig. S1). A total of 1258 unique variants were called, of which 89 (7%) were novel (i.e. not reported in dbSNP). The majority (n=603; 48%) of variants in the call set were classified as intronic variants (Fig. 4.2), and of the variants in coding regions (n=188; 15%), 51% were synonymous (n=98) and

45% were missense variants (n=85) and only 4% were either stop gain, indel or frameshift (n=5) (Fig. 4.2).

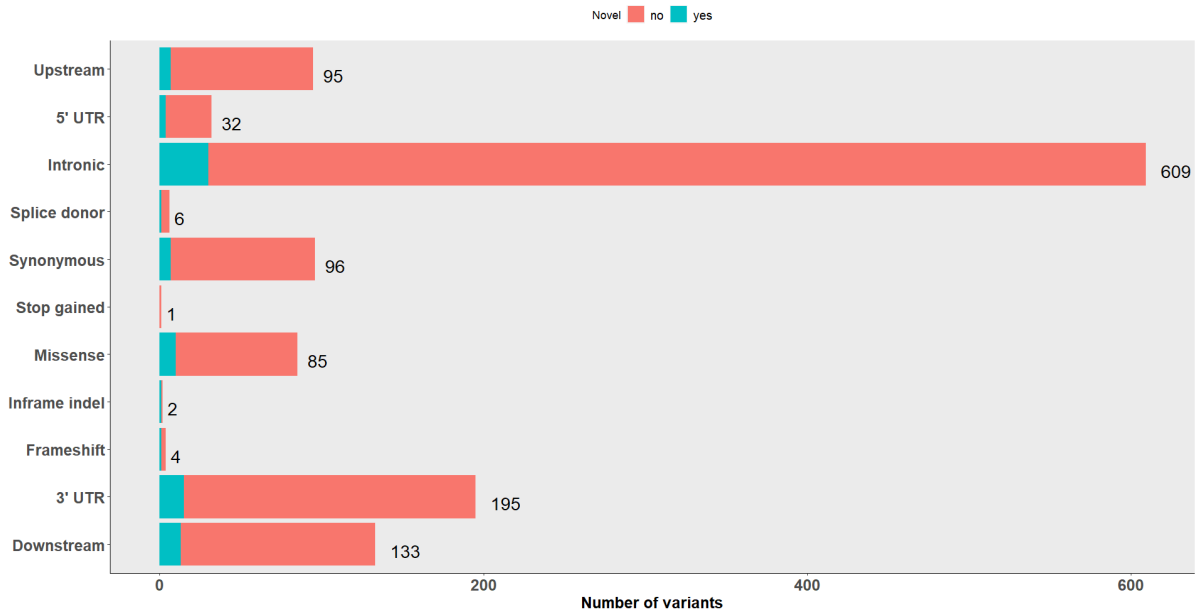


Figure 3. 2: Classification of variants (n=1258) called in the cohort according to Ensembl VEP using the location of the variant. Variants in coding regions further classified by potential consequence. Novel and known variants are distinguished by blue and red shading respectively.

The scores from in silico prediction tools for potentially protein-altering variants indicated that almost half (40/81) of the known variants were predicted to be deleterious by at least one tool (Table 3.2). For the novel variants (n=12) (Table 3.3), 67% were predicted to deleterious by at least one tool.

Only three variants had a consensus deleterious prediction (multiple tools predicting a variant to be deleterious). Missense variant rs2049695991 (*NEDD4L*) and chr4:2898251~A>G (a novel variant in *ADD1*) were predicted to be deleterious by both CADD and REVEL although they were rarely seen in the cohort with an allele frequency less than 1%. The rs2049695991 variant had a CADD score of 32 and a REVEL score of 0.71, and the novel *ADD1* variant had a CADD score of 25.4 and a REVEL score of 0.61. For rs746484082, a multi-allelic frameshift mutation in *WNK1*, each allele was predicted to be deleterious by both CADD and LOFTEE. All splice variants were predicted to be neutral by both CADD and SpliceAI (Table S3.2). Over half of the missense variants (44/85) were predicted to be deleterious by CADD only. The only stop gain variant in *ACE* (COSV52012081) was predicted to be deleterious by CADD.

Table 3. 2: Frequency and functional predictions of known protein-altering variants observed in the cohort

RSID	Gene	Classification	REVEL[1]	CADD[2]	AF (SA)[3]	AF (Africa)[4]

rs6201(A>G)	*HSD3B1*	Missense	0.21	1.682	0.376	0.39
rs6684974(G>A)	*HSD3B1*	Missense	0.25	12.9	<0.01	<0.01
rs587607443(A>G)	*HSD3B1*	Missense	0.25	8.284	0.011	_
rs6205(T>C)	*HSD3B1*	Missense	0.12	0.859	0.426	0.46
rs1047303(C>A)	*HSD3B1*	Missense	0.13	0.025	0.872	0.91
rs2236295(G>T)	***ADO***	**Missense**	**0.05**	**24.1**	**<0.01**	**0.02**
rs10995311(C>G)	*ADO*	Missense	0.09	14.73	0.018	0.04
rs766866164(G>A)	***WNK1***	**Missense**	**0.05**	**17.45**	**<0.01**	**_**
rs11554421(G>A)	***WNK1***	**Missense**	**0.09**	**15.17**	**0.029**	**0.017**
rs971232152(G>C)	*WNK1*	Missense	0.10	7.907	<0.01	_
rs34728563(T>C)	***WNK1***	**Missense**	**0.09**	**20.8**	**0.04**	**0.05**
rs746484082(GA>G)	***WNK1***	**Frameshift**	**NA**	**30**	**0.394**	**<0.01**
rs746484082(GA>GAA)	***WNK1***	**Frameshift**	**NA**	**30**	**0.012**	**<0.01**
rs746484082(GA>GAAAA)	***WNK1***	**Frameshift**	**NA**	**30**	**0.014**	**<0.01**
rs764368160(A>G)	***WNK1***	**Missense**	**0.08**	**16.85**	**0.01**	**_**
rs11833299(A>G)	***WNK1***	**Missense**	**0.15**	**24.6**	**0.021**	**0.03**
rs61736905(T>C)	*WNK1*	Missense	0.04	11.92	0.038	0.05
rs139449198(A>G)	*WNK1*	Missense	0.05	13.03	<0.01	<0.01
rs762367651(C>T)	***WNK1***	**Missense**	**0.08**	**17.49**	**<0.01**	**_**
rs956868(A>C)	*WNK1*	Missense	0.05	7.934	0.834	0.86
rs138216561(A>G)	*WNK1*	Missense	0.09	7.828	<0.01	_
rs368621387(G>A)	*WNK1*	Missense	0.08	0.243	<0.01	_
rs575881382(C>G)	*WNK1*	Missense	0.06	7.9	<0.01	<0.01
rs7955371(G>C)	*WNK1*	Missense	0.12	3.922	0.938	0.95
rs146861951(G>A)	***WNK1***	**Missense**	**0.02**	**18.34**	**<0.01**	**_**
rs748898332(G>C)	***WNK1***	**Missense**	**0.05**	**15.48**	**<0.01**	**_**
rs12828016(G>T)	***WNK1***	**Missense**	**0.01**	**15.64**	**0.514**	**0.55**
rs61747607(A>G)	*GNB3*	Missense	0.19	0	0.019	0.01
rs748642355(G>A)	***TTC6***	**Missense**	**NA**	**16.47**	**<0.01**	**<0.01**
rs999476305(A>G)	***TTC6***	**Missense**	**NA**	**23.1**	**<0.01**	**_**
rs768751920(C>T)	*TTC6*	Missense	NA	0.709	0.00137	<0.01
rs116578389(C>T)	*TTC6*	Missense	NA	11.01	0.058	0.05
rs12896790(T>G)	*TTC6*	Missense	0.05	9.621	<0.01	0.05

rs4901284(C>T)	***TTC6***	**Missense**	**0.07**	**16**	**0.162**	**0.29**
rs17107176(A>T)	*TTC6*	Missense	0.03	0	0.224	0.07
rs61740232(T>C)	*TTC6*	Missense	0.40	**23**	<0.01	<0.01
rs754819517(C>T)	***ALDH1A2***	**Missense**	**0.15**	**19.69**	**<0.01**	_
rs4646626(C>T)	***ALDH1A2***	**Missense**	**0.18**	**17.37**	**0.29**	**0.38**
rs115875978(C>A)	***ALDH1A2***	**Missense**	**0.45**	**24.9**	**0.019**	**<0.01**
rs1272956564(T>C)	*TXNDC11*	Missense	0.08	8.062	<0.01	_
rs144296098(T>A)	***TXNDC11***	**Missense**	**0.16**	**24.4**	**<0.01**	**<0.01**
rs560075677(G>C)	***TXNDC11***	**Missense**	**0.06**	**16**	**<0.01**	**0.02**
rs534277277(G>C)	***TXNDC11***	**Missense**	**0.04**	**23**	**<0.01**	**<0.01**
rs3743588(G>A)	***TXNDC11***	**Missense**	**0.03**	**22**	**0.027**	**0.02**
rs781662574(C>T)	***TXNDC11***	**Missense**	**0.05**	**22**	**0.019**	**<0.01**
rs929252570(T>C)	***TXNDC11***	**Missense**	**0.04**	**16**	**<0.01**	_
rs5736(G>A)	*SCNN1G*	Missense	0.14	1.042	0.041	0.04
COSV55597704(G>C)	***SCNN1G***	**Missense**	**0.13**	**24**	**<0.01**	_
rs532691783(T>TGCTGCC)	*ACE*	Inframe indel	NA	7.649	<0.01	_
rs4317(T>C)	*ACE*	Missense	0.01	0.924	0.276	0.2
rs4318(A>G)	*ACE*	Missense	0.01	6.912	0.257	0.2
COSV52014914(C>G)	*ACE*	Missense	0.10	12.75	<0.01	_
rs3730043(C>T)	***ACE***	**Missense**	**0.22**	**24.7**	**<0.01**	**<0.01**
rs551723440(A>G)	*ACE*	Missense	0.03	13.36	0.027	_
COSV52012081(G>A)	***ACE***	**Stop gained**	**NA**	**48**	**<0.01**	_
COSV52006486(C>A)	***ACE***	**Missense**	**0.45**	**24.7**	**<0.01**	_
rs144751624(T>A)	*ACE*	Missense	0.46	6.743	<0.01	<0.01
rs145349565(C>A)	***ACE***	**Missense**	**0.48**	**24.1**	**<0.01**	**<0.01**
rs4980(G>A)	***ACE***	**Missense**	**0.05**	**23.2**	**<0.01**	**<0.01**
rs4364(C>A)	*ACE*	Missense	0.05	13.31	0.126	0.13
rs12720745(G>A)	*ACE*	Missense	0.06	0.004	<0.01	<0.01
rs61762396(G>A)	*PRKCA*	Missense	NA	7.915	0.038	0.01
rs141376042(G>A)	***PRKCA***	**Missense**	**0.06**	**20.8**	**0.025**	**<0.01**
rs968257095(C>T)	***NEDD4L***	**Missense**	**0.13**	**23.9**	**<0.01**	_
rs767136811(C>T)	***NEDD4L***	**Missense**	**0.03**	**24.1**	**<0.01**	_
rs2049695991(G>T)	***NEDD4L***	**Missense**	**0.71**	**32**	**<0.01**	_

rs111980524(G>A)	*AGTR1*	Missense	0.14	9.103	0.012	<0.01
rs146536048(G>A)	***ADD1***	**Missense**	**0.09**	**17.25**	**0.012**	**<0.01**
rs4963(C>G)	***ADD1***	**Missense**	**0.09**	**18.12**	**0.228**	**0.18**
rs199688227(G>A)	*NOS3*	Missense	0.02	0.331	<0.01	<0.01
rs3918166(G>A)	*NOS3*	Missense	0.05	13.33	0.04	0.02
rs149224629(A>T)	***NOS3***	**Missense**	**0.08**	**22.1**	**<0.01**	**<0.01**
COSV52496818(G>C)	*NOS3*	Missense	0.02	13.28	<0.01	_
rs377756718(G>A)	***NOS3***	**Missense**	**0.20**	**24**	**<0.01**	**_**
rs41377046(G>A)	***NOS3***	**Missense**	**0.24**	**22.3**	**<0.01**	**_**
rs1799983(T>G)	*NOS3*	Missense	0.06	10.07	0.202	0.93
rs145168353(G>A)	***NOS3***	**Missense**	**0.08**	**22.8**	**<0.01**	**_**
rs7792133(G>A)	***NOS3***	**Missense**	**0.14**	**20.8**	**<0.01**	**0.02**
rs369652420(G>A)	***NOS3***	**Missense**	**0.08**	**19.61**	**<0.01**	**<0.01**
rs3918201(G>T)	***NOS3***	**Missense**	**0.10**	**22**	**0.082**	**0.05**

[1] REVEL deleterious criteria: score >= 0.5 = deleterious, score < 0.5 = neutral

[2] CADD deleterious criteria: score >= 15 = deleterious, score < 15 = neutral

[3] Allele frequency in our cohort (n=364)

[4]Allele frequency according 1000 Genomes Project, where data is available (Auton et al., 2015)

Variants with scores denoting deleteriousness according to either CADD or REVEL formatted with bold font

All positions are according to GRCh38

Table 3. 3: Novel protein-altering variants in the Soweto cohort.

Variant	Gene	Classification	Revel[1]	CADD[2]	AF[3]
chr12:754068~A>T	***WNK1***	**Missense**	**0.09**	**20.2**	**0.001374**
chr12:885344~TCTA>T	*WNK1*	Inframe indel	NA	NA	0.001374
chr14:37682826~G>T	***TTC6***	**Missense**	**NA**	**20.3**	**0.001374**
chr14:37841565~A>G	*TTC6*	Missense	0.06	9	0.001374
chr16:11688344~C>T	***TXNDC11***	**Missense**	**0.37**	**25.6**	**0.001374**
chr16:11691304~A>C	*TXNDC11*	Missense	0.04	14.61	0.001374

chr16:11736066~A>T	***TXNDC11***	**Missense**	**0.24**	**28**	0.001374
chr17:63497257~T>C	***ACE***	**Missense**	**0.07**	**23.6**	0.001374
chr3:148741747~AT>A	*AGTR1*	Frameshift	NA	NA	0.001374
chr4:2898251~A>G	***ADD1***	**Missense**	**0.61**	**25.4**	0.001374
chr4:2914981~C>A	***ADD1***	**Missense**	**0.25**	**25.3**	0.001374
chr7:150993821~C>G	***NOS3***	**Missense**	**0.22**	**18.12**	**0.032**

[1] REVEL deleterious criteria: score >= 0.5 = deleterious, score < 0.5 = neutral

[2] CADD deleterious criteria: score >= 15 = deleterious, score < 15 = neutral

[3] Allele frequency (n=364)

Variants with scores denoting deleteriousness according to either CADD or REVEL are indicated in bold.

All positions are according to GRCh38

3.5.3 Variant Association

Linkage disequilibrium pruning removed 553 variants from the call set. None of the independent variants (n=706) was significantly associated with blood pressure control at first follow up, for responders and non-responders, after correction for multiple testing. A 3' UTR variant in *GNB3* (rs1025450259) was significantly associated with change in systolic ($p < 0.001$) and diastolic ($p < 0.001$) blood pressure, between baseline and follow-up (Table S3.2). In individuals treated with HCTZ monotherapy (n=103), none of the variants (n=706) were significantly associated with blood pressure control at first follow-up, after correction for multiple testing, or with change in either systolic or diastolic blood pressure at first follow-up.

We examined the presence of 8 variants which had been previously reported to have an association with HCTZ response in European, American and Asian populations in our study. Variants rs10995311 and rs5186 were rare in our cohort but common in European and Asian populations according to the 1000 Genomes database (Table 3.4).

Table 3. 4: Distribution of variants with previous reported associations with HCTZ response

RSID	Gene	Variant consequence	AF[1] (SA)	AF (1KG_Afr)[2]	AF (1KG_Eur)[2]	AF (1KG_Eas)[2]
rs10995311(C>G)	*ADO*	Missense	0.02	0.04	0.42	0.17
rs5443(C>T)	*GNB3*	Synonymous	0.80	0.82	0.31	0.51
rs5723(C>G)	*SCNN1G*	Synonymous	0.28	0.25	0.22	0.06
rs5729(T>A)	*SCNN1G*	3' UTR	0.28	0.25	0.22	0.06
rs4149601(G>A)	*NEDD4L*	Intron	0.45	0.34	0.37	0.21

rs1008899(G>A)	*NEDD4L*	Intron	0.06	0.06	0.23	0.51
rs5186(A>C)	*AGTR1*	3' UTR	<0.01	0.02	0.28	0.06
rs1799983(T>G)	*NOS3*	Missense	0.20	0.92	0.65	0.87

[1] AF - Allele frequency; SA - this study of South Africans (n=364)

[2]Population allele frequency according to 1000 Genomes Project (1KG) (30x data), Afr – African (African American and Caribbean) ancestry; Eur – European ancestry; Eas – East Asian ancestry (Auton et al., 2015)

All positions are according to GRCh38

3.6 Discussion

This study provides insights into genetic variation in known hydrochlorothiazide (HCTZ) response-related genes in a cohort of treatment-naive hypertensives from Soweto, with a view towards exploring the influence of genetic diversity on hypertension (HT) management in a South African context. HT management is challenging due to its multifactorial nature, influenced by genetic, environmental, and physiological factors. High-income countries report HT control rates of 43% for women and 37% for men, while sub-Saharan Africa and Oceania have the lowest HT control rates globally (Zhou et al., 2021). This disparity is likely influenced by factors such as healthcare accessibility (Tessema et al., 2022; Baazeem et al., 2024), the burden of comorbidities, urbanization (McLennan and Ulijaszek, 2015; Mudie et al., 2019), and socioeconomic disparities (Chan et al., 2008; Antignac et al., 2018). Despite these challenges also being present in South Africa, we observed that 51% of participants (58% for women and 40% for men) in a sub-set of clinics in Soweto achieved control of their HT at the first follow-up visit. However, there are significant knowledge gaps regarding the contribution of genetic variation towards the variability in response to commonly prescribed antihypertensives. Understanding how genetic variation affects HT treatment response is important for developing population-specific pharmacotherapy, particularly for African populations, which are underrepresented in genetic studies.

Despite the immense genetic diversity in continental African populations, they account for only 0.19% of participants in global genome-wide association studies (GWAS) (Mills and Rahal, 2020). Genetic diversity in Africa, characterized by low linkage disequilibrium (LD) and high complex population structure (Yu et al., 2002; Campbell and Tishkoff, 2008; Gomez et al., 2014), presents unique challenges and opportunities for pharmacogenetics research. Using targeted next-generation sequencing, and a custom panel we were able to discover a considerable number of novel variants (7% of the total number of identified variants) in genes linked to HCTZ response and identified 89 novel variants. Twelve of these variants are of interest as they are situated in protein-altering regions and potentially affect function. This was supported by *in silico* tools flagging 8 variants as having a high probability to be deleterious. These findings highlight the need to explore genetic variation and its effect on drug response in continental Africans.

We observed differences in population allele frequencies of key variants previously reported to have an association with HCTZ response. Particularly, rs10995311 and rs5186, are common in European and Asian populations, but rare in our cohort, suggesting possible population-specific differences in genetic contributions to HT medication response. The different definitions of ethnicity complicate the interpretability of allele frequency insights. Resources such as 1000 Genomes Project do not contain data from many populations (including sub-Saharan African) and therefore this limited the comparability of the different variant allele frequencies. While several studies have examined genetic variation in HCTZ response, only one study conducted in America, used blood pressure (BP) control as an outcome and identified (including replication) rs2611316 (in *ALDH1A2*) to be associated with increased risk of uncontrolled BP (Magvanjav et al., 2017). However, this variant was not observed in our cohort, and contrary to findings from other studies, we did not find associations between variants in *ACE, ADD1, ALDH1A2,* or *SCNN1G* (Rysz et al., 2020) with treatment response. We identified an intronic variant in *GNB3* (rs1025450259) associated with a reduction in both systolic and diastolic BP. *GNB3* plays a critical role in BP regulation through its GTPase activity (Bourne et al., 1991), making it a gene of interest for further study. However, the low allele frequency of rs1025450259 complicates its interpretation. Given the complexity of BP regulation (driven by multiple genetic, environmental, and lifestyle factors), it is difficult to pinpoint single or high-effect genetic variants that may impact treatment outcomes.

Our study emphasizes the limitations of current in silico prediction tools, which often disagreed on the consequences of protein-altering variants. The existing predictive tools yield inconclusive results for these African population-specific variants, as seen in our study. African populations are generally underrepresented in databases that variant effect predictive tools use for training prediction algorithms. This may lead to effect predictions which are biased towards represented populations. This challenge of predictive algorithms for treatment outcomes is exacerbated by the high genetic diversity present in African populations which influences the genetic architecture of specific traits (Kessler et al., 2016). Tools which are calibrated to the diverse genetic landscape of African populations may produce more accurate and insightful predictions.

Although all variants met filtering criteria, and the allele depths of singletons were manually inspected on Integrative Genomics Viewer (IGV) (Robinson et al., 2011), the validation of novel variants identified was beyond the scope of the study. We provided *in silico* impact prediction scores as evidence, but technical validation (through Sanger sequencing or replication studies), functional validation (through *in vitro* and *in vivo* studies) and clinical validation is still required to fully understand the impact of the novel variants.

Our candidate gene next generation sequencing approach successfully identified novel variants and highlighted the genetic diversity of continental Africans, underscoring the need for further investment in pharmacogenetic research in Africa. However, the small sample size had by insufficient power to detect treatment response associations with low-effect variants. In addition, the observational nature of the study introduced variability in treatment regimens and lifestyle factors making a case for more clinical drug trials for hypertension in an African setting. A more controlled, larger-scale study with multiple follow-ups and better monitoring

of compliance and lifestyle factors would provide a more robust dataset for investigating the role of genetic variation in HT treatment outcomes among African hypertensives.

3.7 Study highlights

What is the current knowledge on the topic?

There are population differences in responses to hypertension treatment and genetic variants have been associated with this differential response, including to the thiazide diuretic, HCTZ.

What question did this study address?

Whether there is genetic variation in HCTZ-related genes, in the South African population and how it could impact response to treatment.

What does this study add to our knowledge?

The study highlights the occurrence of 8 variants previously associated with HCTZ response in European, American and Asian populations, but did not find them to be significantly associated in our study. The study underscores the rich genetic diversity in continental African populations, in HCTZ-related genes.

How might this change clinical pharmacology or translational science?

The critical, yet underexplored, population specific genetic diversity potentially plays a role in drug response and hypertension management. This study emphasizes the need for continued investment in genetic studies and the development of African-appropriate prognostic tools as an essential approach for advancing personalized medicine through better understanding the role of genetics in creating more equitable and effective hypertension treatments and practises.

3.8 Acknowledgements

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3.9 Author Contributions

Conceptualization, K.N.,D.T.,A.M.,M.R.,C.M.; Methodology, K.N.,D.T.,A.M; Resources, M.R.,C.M.; Writing-original draft , K.N..; review and editing, K.N.,D.T.,A.M.,M.R.,C.M.; Visualization, KN; .; All authors have read and agreed to the final version of the manuscript.

3.10 Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors on request due to privacy restrictions.

3.11 Supplementary materials

Table S3. 1: Candidate gene information and role in hydrochlorothiazide action.

Gene	Location[1]	Gene name	Function	Relevance to HCTZ
ADD1	Chr4:2843844-2930076	Alpha-Adducin 1	Subunit for cytoskeletal protein which binds with high affinity to Ca(2+)/calmodulin and is also a substrate for protein kinase A and C.	Thiazide diuretics work by blocking Na/Cl channel in the kidney, blocking calcium reabsorption and as a result fluid reabsorption
RAD52	Chr12:911736-949694	RAD52 homolog	DNA double-strand break repair and homologous recombination	
WNK1	Chr12:752579-911452	WNK Lysine Deficient Protein Kinase 1	Member of the WNK subfamily of serine/threonine protein kinases	Controls the transport of sodium and chloride ions
NEDD4L	Chr18:58044226-58401539	NEDD4 Like E3 Ubiquitin Protein Ligase	Member of the Nedd4 family of HECT domain E3 ubiquitin ligases	The encoded protein mediates the ubiquitination of multiple target substrates and plays a critical role in epithelial sodium transport by regulating the cell surface expression of the epithelial sodium channel, ENaC
ACE	Chr17:63477061-63498380	Angiotensin I Converting Enzyme	It catalyzes the conversion of angiotensin I into a physiologically	Angiotensin II is a potent vasopressor and aldosterone-

			active peptide angiotensin II.	stimulating peptide that controls blood pressure and fluid-electrolyte balance.
AGTR1	Chr3:148697784-148743008	Angiotensin II Receptor type 1	Receptor for angiotensin II. Mediates its action by association with G proteins that activate a phosphatidylinositol-calcium second messenger system	Angiotensin II is a potent vasopressor hormone and a primary regulator of aldosterone secretion. It is an important effector controlling blood pressure and volume in the cardiovascular system. It acts through at least two types of receptors. AGTR1 is one of them.
YEATS4	Chr12:69359710-69390870	YEATS Domain Containing 4	Transcription factor	
NOS3	Chr7:150688144-150711687	Nitric oxide synthase 3	Synthase which produces Nitric oxide from L-arginine	Implicated in vascular smooth muscle relaxation through a cGMP-mediated signal transduction pathway
PRKCA	Chr17:64298926-64806862	Protein Kinase C Alpha	Member of family of serine- and threonine-specific protein kinases that can be activated by calcium and the	Its signalling affects mechanisms of antihypertensive medications

			second messenger diacylglycerol	
GNB3	Chr12:6949375-6956557	G Protein Subunit Beta 3	Subunit of guanine nucleotide-binding proteins (G proteins) - which are involved as a modulator or transducer in various transmembrane signalling systems	GTPase activity crucial for controlling blood pressure.
ALDH1A2	Chr15:58245622-58358121	Aldehyde Dehydrogenase 1 Family Member A2	Enzyme that catalyzes the synthesis of retinoic acid (RA) from retinaldehyde. Retinoic acid, the active derivative of vitamin A (retinol), is a hormonal signalling molecule that functions in developing and adult tissues	Retinoic acid is an active compound in kidney physiology which is the site of action of HCTZ
TXNDC11	Chr16:11772943-11836648	Thioredoxin Domain Containing 11	Involved in redox reactions and protein folding in the endoplasmic reticulum	
TTC6	Chr14:38080456-38510647	Tetratricopeptide Repeat Domain 6	Believed to play a role in protein-protein interactions due to its tetratricopeptide repeat domains	
SCNN1G	Chr16:23194040-23228200	Sodium Channel Epithelial 1 Gamma Subunit	Component of the epithelial sodium channel (ENaC) which is critical for sodium balance and blood pressure regulation	Involved in the reabsorption of sodium in the kidneys

HSD3B1	Chr1:120049826-120057681	Hydroxy-Delta-5-Steroid Dehydrogenase, 3 Beta- And Steroid Delta-Isomerase 1	Involved in steroid hormone biosynthesis	
ADO	Chr10:64564516-64568239	2-Aminoadipate-Semialdehyde Dehydrogenase	Involved in lysine degradation and the metabolic pathway of alpha-aminoadipate	
[1] Based on hg19 build of the UCSC genome browser				



Figure S3. 1: Average depth of sequenced amplicons per gene for the cohort.

Table S3. 2: Association results of genetic variants with the change in blood pressure between baseline and follow-up.

Systolic blood pressure						
SNP ID	Location	Gene	Allele	Beta (SE)	95% CI	p-value
rs1025450259	chr12:6847211	*GNB3*	G	-86.84(23.5)	-134.1: -41.99	<0.001
Diastolic blood pressure						
rs1025450259	chr12:6847211	*GNB3*	G	-53.97(10.15)	-73.87:34.08	<0.001

Table S3. 3: In silico impact predictions for splice variants (n=6) according to SpliceAI and CADD.

Variant	RSID	Gene	CADD[1]	SpliceAI[2]	AF[3]
chr12:883063~C>T	rs304032084	WNK1	9.547	0.01	0.026
chr12:894638~A>G	rs72650758	WNK1	14.82	0.01	0.022
chr12:900676~A>G	NA	WNK1	10.91	0.02	<0.01
chr12:927139~G>A	rs755796257	WNK1	0.081	0.15	<0.01
chr17:63494475~C>T	rs12720737	ACE	0.0387	0.07	0.10
chr18:58329132~G>A	rs199959002	NEDD4L	9.451	0.01	0.03

[1]CADD deleterious criteria: score >= 15 = deleterious, score < 15 = neutral

[2] SpliceAI deleterious criteria: score >= 0.5 = deleterious, score < 0.5 = neutral

[3]Allele frequency (n=364)

Table S3. 4: Novel variants identified through candidate gene NGS in black South African hypertensives.

Variant[1]	Allele frequency [2]	Gene	Classification [3]
chr1:119513747~TG>T	0.001374	HSD3B1	Intronic
chr1:119514853~C>T	0.005495	HSD3B1	3' UTR
chr10:62806671~A>G	0.008242	ADO	3' UTR
chr10:62807233~A>G	0.001374	EGR2	Downstream
chr10:62807700~A>G	0.001374	EGR2	Downstream
chr10:62808046~G>T	0.002747	EGR2	Downstream
chr12:752857~C>G	0.002747	WNK1	5' UTR
chr12:753141~G>C	0.001374	WNK1	5' UTR
chr12:754068~A>T	0.001374	WNK1	Missense
chr12:860947~G>A	0.001374	WNK1	Intronic
chr12:883287~AAAAT>A	0.001374	WNK1	Intronic
chr12:885344~TCTA>T	0.001374	WNK1	Inframe indel
chr12:896211~G>A	0.001374	WNK1	Synonymous
chr12:900676~A>G	0.002747	WNK1	Splice donor
chr12:908041~C>A	0.001374	RAD52	Downstream
chr12:909038~C>T	0.001374	RAD52	Downstream
chr12:909041~T>C	0.001374	RAD52	Downstream
chr12:911087~A>G	0.001374	RAD52	Downstream
chr12:930241~T>G	0.001374	RAD52	Intronic
chr12:931171~G>C	0.002747	RAD52	Intronic
chr12:949633~A>C	0.001374	RAD52	5' UTR
chr12:949681~G>T	0.001374	RAD52	5' UTR
chr12:989928~AGGCT>A	0.001374	ERC1	Upstream
chr12:6841498~A>G	0.001374	GNB3	Intronic
chr12:6844030~C>T	0.001374	GNB3	Intronic
chr12:6847400~T>C	0.004121	GNB3	Downstream
chr14:37595533~G>A	0.03	FOXA1	Upstream
chr14:37595533~G>T	0.42	FOXA1	Upstream
chr14:37606745~A>G	0.004121	TTC6	Upstream
chr14:37682826~G>T	0.001374	TTC6	Missense
chr14:37714888~T>G	0.001374	TTC6	Intronic
chr14:37751178~A>G	0.004121	TTC6	Intronic
chr14:37751312~C>A	0.001374	TTC6	Intronic
chr14:37807341~T>G	0.001374	TTC6	Intronic
chr14:37823986~AG>A	0.004121	TTC6	Intronic
chr14:37841565~A>G	0.001374	TTC6	Missense
chr15:57953839~G>A	0.001374	ALDH1A2	3' UTR
chr15:57954206~G>A	0.001374	ALDH1A2	3' UTR
chr15:57954250~A>C	0.001374	ALDH1A2	3' UTR
chr15:57954321~T>A	0.001374	ALDH1A2	3' UTR

chr15:58010581~T>C	0.001374	ALDH1A2	Intronic
chr15:58014041~A>ATGTCTCCTCGC	0.001374	ALDH1A2	Intronic
chr16:11684097~C>A	0.001374	TXNDC11	Intronic
chr16:11688344~C>T	0.001374	TXNDC11	Missense
chr16:11691304~A>C	0.001374	TXNDC11	Missense
chr16:11700354~G>C	0.001374	TXNDC11	Intronic
chr16:11736066~A>T	0.001374	TXNDC11	Missense
chr16:11742626~C>A	0.001374	TXNDC11	Synonymous
chr16:11742879~C>A	0.005495	TXNDC11	Upstream
chr16:23186559~A>G	0.001374	SCNN1G	Synonymous
chr16:23192297~T>C	0.001374	SCNN1G	Intronic
chr17:63482470~A>C	0.001374	ACE	Upstream
chr17:63484435~A>C	0.001374	ACE	Upstream
chr17:63493587~C>T	0.001374	ACE	Synonymous
chr17:63497257~T>C	0.001374	ACE	Missense
chr17:66742593~G>T	0.001374	PRKCA	Intronic
chr17:66805100~C>T	0.001374	PRKCA	3' UTR
chr17:66806024~TTTCTTCCAGCCATTGTGGCATTGGCCTTTCCAG>T	0.001374	PRKCA	3' UTR
chr17:66806503~G>A	0.001374	PRKCA	3' UTR
chr17:66809205~A>T	0.001374	PRKCA	3' UTR
chr17:66809340~T>C	0.001374	PRKCA	3' UTR
chr18:58047450~A>G	0.001374	NEDD4L	Intronic
chr18:58166066~A>C	0.001374	NEDD4L	Intronic
chr18:58195702~A>G	0.001374	NEDD4L	Intronic
chr18:58221685~AGATTT>A	0.001374	NEDD4L	Intronic
chr18:58329004~C>T	0.001374	NEDD4L	Synonymous
chr18:58396734~C>CA	0.001374	NEDD4L	Downstream
chr18:58397791~T>C	0.001374	NEDD4L	Downstream
chr18:58398125~G>A	0.001374	NEDD4L	Downstream
chr18:58400368~TG>T	0.002747	NEDD4L	Downstream
chr3:148730091~T>TCCAACAATGTTTTGCAC	0.002747	AGTR1	Intronic
chr3:148741747~AT>A	0.001374	AGTR1	Frameshift
chr3:148742311~G>A	0.001374	AGTR1	3' UTR
chr3:148742464~A>C	0.001374	AGTR1	3' UTR
chr4:2844313~G>GGCCT	0.001374	ADD1	Intronic
chr4:2844629~G>A	0.001374	ADD1	Intronic
chr4:2867833~TCTGGAGTCAGGCAG>T	0.001374	ADD1	Intronic
chr4:2898251~A>G	0.001374	ADD1	Missense
chr4:2898360~C>T	0.001374	ADD1	Intronic
chr4:2907959~A>T	0.001374	ADD1	Intronic

chr4:2914981~C>A	0.001374	ADD1	Missense
chr4:2930144~C>G	0.001374	ADD1	Downstream
chr7:150993821~C>G	0.032	NOS3	Missense
chr7:151001442~G>A	0.001374	NOS3	Intronic
chr7:151002274~T>C	0.001374	NOS3	Synonymous
chr7:151010617~G>A	0.006868	NOS3	Synonymous
chr7:151010998~G>C	0.002747	NOS3	Intronic
chr7:151014520~T>C	0.001374	NOS3	3' UTR
chr7:151014540~G>T	0.001374	NOS3	3' UTR

[1] Variants denoted as chromosome:position~reference allele>alternate allele
[2] n= 364 participants
[3] According VEP annotations

Table S3. 5: Association statistics and information of top 5 variant hits from a linear regression analysis of the change in diastolic blood between baseline and follow up in response to treatment.

Variant	Gene	Consequence [1]	Allele Frequency [2]	*z* statistic [3]	Unadjusted p-value	Adjusted p-value
rs1025450259	*GNB3*	3' UTR	0.0027	-5.318	1.86×10^{-7}	0.000163
rs147198879	*NEDD4L*	Intronic	0.012	3.317	0.001	0.88
rs1219214653	*ADD1*	3' UTR	0.0014	-3.293	0.0011	0.96
rs116578389	*TTC6*	Missense	0.06	-2.99	0.0030	1
rs1010546	*PRKCA*	Intronic	0.04	2.976	0.0031	1

[1] As predicted by VEP
[2] n = 364 participants
[3] Computed as *Beta /* Standard error

Table S3. 6: Association statistics and information of top 5 variant hits from a linear regression analysis of the change in systolic blood between baseline and follow up in response to treatment.

Variant	Gene	Consequence [1]	Allele Frequency [2]	*z* statistic [3]	Unadjusted p-value	Adjusted p-value
rs1025450259	*GNB3*	3' UTR	0.0027	-5.311	1.93×10^{-7}	0.0002
rs148701233	*TXNDC11*	Intronic	0.0027	-3.904	0.00011	0.09968
rs1219214653	*ADD1*	3' UTR	0.0014	-3.747	0.00020	0.1842
chr4:2898360~C>T	*ADD1*	Intronic	0.0014	-3.262	0.00121	1
chr14:37682826~G>T	*TTC6*	Missense	0.0014	-3.262	0.00121	1

[1] As predicted by VEP
[2] n = 364 participants
[3] Computed as *Beta* / Standard error

Table S3. 7: Association statistics and information of top 5 variant hits from a logistic regression analysis of the BP control at follow up in response to treatment.

Variant	Gene	Consequence [1]	Allele Frequency [2]	*z* statistic [3]	Unadjusted p-value	Adjusted p-value
rs3730009	*NOS3*	Intronic	0.143	-3.331	0.008656	0.7834
rs76511847	*ADO*	3' UTR	0.058	-2.939	0.003295	1
rs3204690	*ALDH1A2*	3' UTR	0.313	--2.437	0.01483	1
rs7218270	*MicroRNA634*	5' UTR	0.102	-2.393	0.01673	1
rs5445	*GNB3*	3' UTR	0.305	-2.383	0.01715	1

[1] As predicted by VEP
[2] n = 364 participants
[3] Computed as *Beta* / Standard error

Table S3. 8: Comparison of association p-values of 8 variants previously associated with response to HCTZ, in non-African populations.

RSID	Gene	Variant consequence	AF[1]	Association (SBP)[2]		Association (DBP)[3]		Association (BP control)[4]		Previous association
				Unadjusted p-value	Adjusted p-value	Unadjusted p-value	Adjusted p-value	Unadjusted p-value	Adjusted p-value	
rs10995311(C>G)	*ADO*	Missense	0.02	_	_	_	_	_	_	(Iniesta et al., 2019)
rs5443(C>T)	*GNB3*	Synonymous	0.80	_	_	_	_	_	_	(Turner et al., 2000)
rs5723(C>G)	*SCNN1G*	Synonymous	0.28	_	_	_	_	_	_	(Turner et al., 2005)
rs5729(T>A)	*SCNN1G*	3' UTR	0.28	_	_	_	_	_	_	(Turner et al., 2005)
rs4149601(G>A)	*NEDD4L*	Intron	0.45	_	_	_	_	_	_	(McDonough et al., 2013b)
rs1008899(G>A)	*NEDD4L*	Intron	0.06	0.6165	1	0.6877	1	0.6061	1	(McDonough et al., 2013a)
rs5186(A>C)	*AGTR1*	3' UTR	<0.01	0.1545	1	0.1093	1	0.054	1	(Frazier et al., 2004)
rs1799983(T>G)	*NOS3*	Missense	0.20	_	_	_	_	_	_	(Turner et al., 2003)

[1] AF - Allele frequency; (n=364)

[2] Linear regression analysis of change in systolic blood pressure between baseline and follow up; _ - variants pruned due to linkage disequilibrium

[3] Linear regression analysis of change in diastolic blood pressure between baseline and follow up; _ - variants pruned due to linkage disequilibrium

[4] Logistic regression analysis of BP control outcome at follow up; _ - variants pruned due to linkage disequilibrium

Chapter 4: Discussion and Conclusions

This PhD study aimed to assess HT treatment practices at primary health care level in Soweto, South Africa and investigate the genetic variation in HCTZ-related genes, in continental Africans and its influence on treatment response. This final chapter examines the strengths and limitations of the study and highlights the research themes emerging from the study.

4.1 Summary of key findings

4.1.1 Objective 1: Hypertension treatment practices and outcomes at health community centres in urban Soweto, South Africa

- The mean age of the participants was 49 years and 60% of the participants were women.
- Only 65% of patients recruited into the study returned for at least one follow-up visit.
- Most patients received a single drug (40%) or dual therapy (53%) after the initial HT diagnosis.
- HCTZ (thiazide diuretic) and Amlodipine (calcium channel blockers) were the most prescribed medications
- The mean systolic and diastolic blood pressure of the participants was lower at first follow-up regardless of the number of medications prescribed.
- Half of the participants (51%) had controlled blood pressure at first follow-up
- There were more females (58%) with controlled blood pressure compared to males (40%)
- Treatment strategy (number and type of drugs) did not influence blood pressure control outcomes as expected. Instead, the treatment clinic seemed to play a role.

4.1.2 Objective 2 and 3: Genetic Variation in Hydrochlorothiazide-Response Related Genes among Hypertensive Individuals in Soweto, South Africa

- A custom panel of 16 genes, chosen through leveraging PharmGKB entries, was designed to target coding regions, intron-exon boundaries and regulatory regions for variants which influence treatment response.
- A total of 1 258 variants were identified. Of these, 7% were novel and 15% were in coding regions.
- In silico prediction tools flagged 51% of known variants and 67% of the novel variants, as potentially deleterious.
- Significant association between rs1025450259 (*GNB3*) and reduction in systolic and diastolic BP (p-value < 0.001) was observed, but the low allele frequency (<1%) decreases confidence in the association.

- Of the eight variants previously associated with HCTZ response in European, Asian and American populations, rs10995311 and rs5186 were rare in our cohort but common in European and Asian populations.

4.2 Emerging Research Themes

4.2.1 Chronic disease management challenges at primary health care level

We conducted an observational study from 2019 to 2023 at seven community health centres in Soweto. We observed that community health centres are often the first point of call for "western style" health care seeking in this setting. We did not explore the role of traditional healing in health seeking behaviour and limited our investigation to people attending the clinics. The clinic nurses screened and, in consultation with the clinical doctors, diagnosed patients with a wide range of symptoms. We also observed that point-of-care blood pressure and blood glucose measurements were taken for each patient during screening. This shows that there is a focus on hypertension and diabetes at primary health care level in Soweto. It is worth noting that the participants recruited into the study were previously undiagnosed and treatment-naive or had not used anti-hypertensive medication for the prior six months. It has been previously reported that undiagnosed hypertension in sub-Saharan Africa was as high as 41% in women and 51% in men (Zhou et al., 2021). Similarly, in the Africa Wits-Indepth Partnership for Genomic research (AWI-GEN) study, over 60% of men and women in Soweto, with hypertension, were not aware of their condition (Gómez-Olivé et al., 2017). The challenges of the way of life in Soweto may be contributing to the high prevalence of undiagnosed hypertension (and other chronic diseases) prevalent in the community. Socio-economic stressors such as unemployment and high levels of poverty are prevalent in Soweto and stress is a major risk factor for hypertension (Kulkarni et al., 1998; Spruill, 2010; Liu et al., 2017). Additionally, these socio-economic factors lead to generally poor dietary patterns and unhealthy habits such as smoking and alcohol use (Boua et al., 2021). Soweto was the site in the AWI-Gen study with the highest smoking and alcohol consumption rates, with up to 71% of men surveyed, reported to drink alcohol (Gómez-Olivé et al., 2017). Food security has previously been found to be an important determinant for diet quality amongst women in Soweto (Kehoe et al., 2021). All these factors compound the risk for undiagnosed hypertension.

Despite there being 23 primary healthcare clinics and 2 public hospitals in Soweto (Adedini et al., 2020), residents often cannot afford to visit the facilities regularly. An American study found that people without routine and regular access to health care were 20% more likely to be unaware of their hypertension compared to those with access (Calixte et al., 2024). Our study suggests that this may be even higher for a community such as Soweto, which has poor preventative health practices, largely driven by, among other factors, poverty. Less than 10% of adults aged over 20 years, in the Soweto region, have a higher education (Bonner and Segal, 1998) and therefore health literacy in the community is likely to be low. This can contribute to an underestimation of the importance of regular blood pressure monitoring.

The long-term health impact of undiagnosed hypertension in Soweto has yet to be investigated. We postulate that it leads to higher rates of severe complications like stroke, heart disease and kidney failure as patients seem to seek help only when they experience

advanced complications. This was supported in discussions with health care practitioners at two of the sites of our study during feedback sessions. Undiagnosed hypertension and late-stage complications result in a greater cost and strain on the healthcare system especially when emergency care or intensive treatment becomes necessary. The direct healthcare costs associated with hypertension in South Africa were estimated at ZAR 10.1 billion in the year 2020 (Kohli-Lynch et al., 2022), which is over 40% of R25 billion budgeted nationally for all communicable and non-communicable diseases (Republic of South Africa, 2023). This is indicative of a system which may already be stretched thin.

The complications related to undiagnosed hypertension may have also contributed to the treatment practices we observed in the study. There was no clear pattern to how medication type and dosage were prescribed to patients. The treatment practices, in terms of the number and class of drugs prescribed, also differed between different facilities. The evidence-based treatment guidelines published for hypertension clearly recommend the criteria for the class and number of medications to be prescribed to patients but also highlight the importance of prioritizing complications such as target organ damage and comorbidities. The discrepancies in the treatment guidelines and the practices we observed at primary health care level in Soweto suggest that complications and comorbidities play a large role in the way hypertension is clinically managed in the community.

4.2.2 Conducting budget-restricted genetic studies

The differences, advantages and disadvantages of candidate gene studies and GWAS have been discussed in chapter 1. We opted for the candidate gene approach it was the more affordable option. The GWAS approach would have cost more than four times the cost of the candidate gene approach. Selecting the candidate genes is an important step for the success of this approach. The results and insights discussed in chapter 3 show the potential of candidate gene approach to produce insights. Using this approach, we were able to: firstly, confirm the presence (or absence) of previously reported variants across the European, American and Asian populations in continental Africans and secondly, we identified novel variation present in continental Africans, in candidate genes.

We used variant effect predictor (VEP) annotations to understand the predicted biology of the variants. We adopted deleterious-prediction scores from in silico tools to infer the potential impact of the variants on the protein product. Finally, we used chi-squared test models to test the variants for association with treatment outcomes.

There were substantially more variants in non-coding regions compared to coding regions with the majority of the variants being intronic. Intron-exon border regions (100 base pairs either side of an exon) contributed the most variants in the call set. Conservation of genomic sequence is linked to functionality (Bejerano et al., 2004). However, this phenomenon depends on chromosome, region and organism. Non-coding DNA, previously believed to not have a function, is actually responsible for RNA processing, mRNA stability and transcription regulation (Shanmugam et al., 2017). There is also growing evidence of the role of non-coding DNA and its influence on hypertension. The expression levels of certain micro RNAs can be used as biomarkers for detecting hypertension (Jusic and Devaux, 2019). Long non-coding RNAs and circular RNAs have also been revealed to contribute to the pathophysiology of

hypertension (Ghafouri-Fard et al., 2022). Although we did not further investigate the non-coding region variants which we identified in our study, their potential role in hypertension response remains an interesting unexplored question.

For the variants in protein coding regions, the majority were synonymous and missense variants. We used common in silico prediction tools to try and determine the impact of the missense variants on the protein product. We found that there were many options for in silico prediction tools, each using different methods and supporting information. This led to different predictions for the same variant. Additionally, in silico tools do not have the ability to definitively label a variant as deleterious or not, instead they give scores on a scale therefore calculating how likely a variant is to be deleterious. Currently there is no in silico tool which can substitute for laboratory-based or in vivo functional studies. However, there is ongoing progress in this field. AlphaFold is an example of how emerging techniques such as deep learning, can greatly improve our ability to predict the impact of variants in coding regions (Jumper et al., 2021). Deep mutational scanning is another promising method for assigning phenotypes to genotypes using model organisms (Wei and Li, 2023).

4.2.3 Specifying treatment outcome for HT association studies

The observational study conducted as part of objective 1 allowed us to stratify study participants into two groups: those with controlled blood pressure in response to treatment at first follow up and those with uncontrolled blood pressure at the same timepoint. The individuals in these two groups were used as the controls and cases for our association analysis, respectively. Our hypothesis was that variants that are significantly overrepresented in the case group contribute to poor hypertension control at first follow up, in response to treatment. We did not find any variants to be significant in this association study. The outcome of BP control is important because it marks the blood pressure threshold where the risk of poor cardiovascular and organ outcomes is minimized (James et al., 2014; Seedat et al., 2014). Due to the lack of longitudinal data, we were limited to defining control using blood pressure measured at one time point, following treatment. This means that we were unable to mitigate the effect of biases, such as white coat hypertension, diurnal (daily) fluctuations and other exogenous factors, which are aggravated when only testing BP at a single time point. Furthermore, the days between the baseline visit and follow up visit for individuals in both the case and control groups were very heterogenous, introducing a temporal bias which we could not control for. The individuals in the case and control groups were also on different number, class and doses of medications, which undoubtedly contributed to their blood pressure control status at first follow up. These confounding variables were a major limitation to our ability to detect genetic variants contributing to response to hypertension treatment, regarding control of blood pressure. This is also related to the challenge of blood pressure control as an endpoint outcome for genetic studies. To date, only one study has investigated genetic association with blood pressure control (Magvanjav et al., 2017) and the investigators experienced similar limitations in their study. Where more longitudinal data is available, it would be possible to investigate genetic variants associated with maintenance of blood pressure control over time. This would mitigate the biases introduced by assessing blood pressure control at a single time point.

As part of sensitivity analyses, we conducted an association study on the same case-control group with change in blood pressure, between baseline and follow-up, as the outcome. This is the more common practise for identifying genetic variants associated with treatment response. We identified one variant, rs1025450259, which was significantly associated with a decrease in systolic and diastolic blood pressure at follow up. rs1025450259 is a 3' prime untranslated region variant in the gene *GNB3*. The G-protein β3 subunit encoded for by the gene, has GTPase activity which is important for sodium processing and hypertension (Siffert et al., 1998). rs1025450259 has not been previously associated with hypertension or any other disease. In our study, although the variant reached the significance threshold, there was not enough evidence to support its influence on treatment response. rs5443 is a synonymous variant in the same gene (*GNB3)* which has been previously associated with risk of hypertension (Siffert, 2003) and obesity (Siffert et al., 1999) and response to calcium channel blockers (Zhang et al., 2016) and thiazide diuretics (Schelleman et al., 2006). This adds additional support to suggest that the *GNB3* gene is involved in the pathway of many conditions and a good candidate for therapeutic intervention.

4.3 Limitations

4.3.1 Sample size

The biggest limitation of our study was the small sample size which reduces statistical power to detect associations with small to moderate effects (Serdar et al., 2020). The study is therefore susceptible to false-negative results and could incorrectly conclude that specific variants had no effect. The small sample size also limited the generalizability of our findings. We found unique treatment practises and described their effects on treatment outcomes however, our cohort was not representative of the broader population within the city, province or country. We, therefore, cannot extrapolate our findings and recommendations to other populations or contexts. As discussed above, hypertension and its management at primary health care level in Soweto, is complex and many factors contribute to diagnosis, treatment and outcomes. The small sample size of our study made it difficult to account for confounding variables that could have influenced the detection of possible genetic variations associated with treatment response.

4.3.2 Study design

The lack of longitudinal data and the heterogeneity of follow up periods between the studied timepoints was another major limitation. Maintenance of hypertension control is crucial for reducing the risk of stroke and heart disease and is the goal of hypertension management. We therefore could not assess the effectiveness of treatment regarding reducing mortality and morbidity as we did not have sufficient data. However, we showed the real-life challenges for hypertension management at primary health care level especially regarding continuity of care.

The observational study design also meant that we had no control over the nature of the intervention (drug type, dose and time to the next visit). Clinical trials with very specific approaches and standard operating procedures are necessary to minimise confounding variables when aiming to understand the response to specific treatments (Kupersmith and Jette, 2023; Zoh et al., 2023). In our study we noted the different treatments prescribed by

clinicians at the different clinics for similar clinical features. Our study was therefore influenced by clinician preferences and possibly by the "culture" for prescription which could differ from clinic to clinic.

4.3.3 Absence of adherence data

The biggest contributor to poor health outcomes for chronic conditions is long-term nonadherence (Sabaté and Organization, 2003). Because many factors contribute to hypertension, adherence is especially important for hypertension management as it facilitates blood pressure regulation in response to any triggers which the patient may be exposed to. There are many methods for measuring adherence including pill counts, therapeutic drug monitoring, biological assays, electronic monitoring and patient interviews (Anghel et al., 2019). The observational design of our study limited our ability to monitor adherence. We had planned to collect blood samples for measuring adherence in participants who did not have controlled blood pressure at subsequent follow up periods. However, the sample needed to be flash frozen immediately after collection and this was logistically challenging at the clinics, so we could not collect an adequate number of samples. We anticipate that adherence played a major role in treatment outcomes as poor adherence has been reported in the region for both communicable (Hornschuh et al., 2017) and non-communicable diseases (Ruf et al., 2010). Financial strain, food insecurity and low income have been associated with poor adherence and bad health outcomes for hypertension and cardiovascular patients (Osborn et al., 2017; Lee et al., 2019; Wilder et al., 2021). The same socioeconomic factors plague the Soweto community and likely contribute to poor adherence reported in the community.

4.3.4 Candidate gene approach

The major limitation of using this approach was that it focused on a selection of genes based on prior knowledge. The study was therefore restricted by this selection bias and could not identify novel genes or unexpected genetic variants in genes outside of the chosen candidates. This is particularly important for hypertension, a disease with a complex aetiology and polygenic influences. This limitation was aggravated by the observation that study participants were often treated with other medications beside (or in combination) with HCTZ. This means that many key genetic variants in genes relevant to other medications were potentially overlooked. Additionally, the candidate gene approach limited our ability to discover new biological pathways and mechanisms underlying the response to hypertension treatment in continental Africans.

4.4 Future studies

This study has identified several inconsistencies with national policy guidelines and differences at different clinics in hypertension management at primary health care level, which future studies can address. Such interventions could help researchers better understand the contextualized prevalence and treatment practises for diverse populations such as those we observed in Soweto. Possible future areas for investigation are listed below:

- **Awareness and determinants of lack of preventative care in resource constrained communities**

The alarming rate of undiagnosed hypertension observed in the study raises the question of why preventative care is so rare in the community. A study exploring whether this behaviour is driven mainly by the lack of resources and/or access to healthcare, or whether low awareness of the dangers of not getting regular health assessments is a key factor, could inform potential interventions. Such a study could look at different perspectives from different stakeholders. It would be interesting to know what the health care practitioners perceive to be the main causes of the high burden of hypertension and how that compares to the views of the community.

- **Long-term assessment of the outcomes in diagnosed hypertensives from diverse communities such as Soweto**

Our study provided a "snapshot" of hypertension management at primary health care level. A multi-year longitudinal study assessing the mortality and long-term effects of hypertension in this community would be important to accurately describe the long-term impact of hypertension and interventions on the community. Such a study would also be an excellent opportunity to assess current treatment recommendations and their effectiveness in South African populations. The relationships established with the Soweto clinics and the Johannesburg Health District provide an opportunity for building larger cohorts with carefully recorded co-morbidities in order to relate genetics to more accurate hypertension phenotypes and defined sub-phenotypes.

- **Genome-wide association study on continental Africans undergoing treatment for hypertension**

Our study has shown that there is unique and undiscovered genetic variation in continental Africans and its contribution to treatment response remains unknown. A genome-wide association study with a large sample size of continental Africans from different African regions would help to address this knowledge gap. Small-scale studies such as this one can also leverage large and publicly available datasets (e.g. UK Biobank or the ALL of Us study) as a tool for assessing clinically relevant associations. In addition, response the different treatment should be assessed. Such studies could be used for replication and confirmation of previously reported variants associated with treatment response. This would be a big step towards personalized medicine in relation to HT treatment in Africa.

- **Pharmacogenomics-driven HT treatment**

Pharmacogenomics testing is already being utilized globally in the treatment of other conditions such as cancer (Xu et al., 2024). There is currently no evidence of a clinically successful pharmacogenomics driven approach for HT treatment in Africa. The complex nature of hypertension is a major obstacle for pharmacogenomics-driven treatment as the current literature on genetic variants associated with treatment response often do not distinguish between the various forms of hypertension. Additionally, the main goal of HT treatment is to reduce the risk of conditions such as chronic kidney disease, heart failure and heart disease and there is no validated evidence of pharmacogenomic approaches which significantly reduce this risk. Future studies therefore should aim to address this knowledge gap by designing clinical studies which investigate the role of genetics in treatment response

of the different types of HT and treatment, and the role of genetics in reducing the risk of HT-related chronic conditions. For example, a genetic risk score based on 44 SNVs was reported to be predictive of survival benefit from beta-blocker exposure in European ancestry individuals with heart failure (Lanfear et al., 2020).

The development of pharmacogenomic-driven methods of HT treatment in Africa and globally requires more robust data compared to other conditions. Integrating multiple “omic” and environmental data can potentially lead to the discovery of more accurate associations of these factors and treatment response. The rapid development of machine learning and artificial intelligence methods allows for the creation of these integrated risk models. Several studies have attempted to use these methods to develop integrated risk models for incident hypertension with varying success (Du et al., 2023; Cai et al., 2024; Schjerven et al., 2024). Future studies can therefore use similar approaches to create risk models which incorporate different forms of data to predict outcomes in response to treatment, leveraging large databases such as the UK Biobank and emerging initiatives in Africa such as the South African 100,000 genomes project.

4.5 Conclusion

In conclusion, this study demonstrated that hypertension management at primary health care level, is complicated and reflects the diversity and challenges of living in Soweto. Despite these challenges, more than half of the participants had controlled blood pressure at the first follow up visit after an initial diagnosis and prescribed medication. The factors contributing to this successful response to treatment, higher than in most reported studies, remain unknown. The days between the visits did not appear to play a role and medical adherence was not explored. This shows that the primary health care practitioners in the community understand the hypertension needs of their patients and are performing admirably at managing hypertension and its associated risks. Importantly, the long term sustained blood pressure control needs to be investigated. There is rich and undiscovered genetic variation in continental Africans across genes relevant for response to HT medication. Dedicating resources to exploring the pharmacogenetic diversity among Africans, and other underrepresented populations remains an important step for a future of tailored treatment for hypertension informed by population-specific characteristics.

Consolidated references

Automatic citation updates are disabled. To see the bibliography, click Refresh in the Zotero tab.

Appendices

Appendix A: Ethics Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO:	Mr K Nyamupangedengu Faculty of Health Sciences Sydney Brenner Institute for Molecular Bioscience Medical School University E-mail: nyamupakuda@gmail.com
CC:	Supervisor: Professors M Ramsay and C Masimirembwa; Dr A May <Michelle.Ramsay@wits.ac.za> and <HREC-Medical Research Office@wits.ac.za>
FROM:	Mr Iain Burns Human Research Ethics Committee (Medical) Tel: 011 717 1252 E-mail: Iain.Burns@wits.ac.za
DATE:	2022/03/10
REF:	R14/49
PROTOCOL NO:	**M220286** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)
PROJECT TITLE:	*Genetic impact of first-line treatment to hypertension in Black South Africans*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

WITWATERSRAND
JOHANNESBURG

R49 Mr K Nyamupangedengu

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M220286

NAME: (Principal Investigator)	Mr K Nyamupangedengu
DEPARTMENT:	Faculty of Health Sciences Sydney Brenner Institute for Molecular Bioscience Medical School University
PROJECT TITLE:	*Genetic impact of first-line treatment to hypertension in Black South Africans*
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-study under M201143
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Professors M Ramsay and C Masimirembwa; Dr A May
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2022/03/10

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **February** and therefore reports and re-certification will be due in the month of **February** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

03/11/2022
Date

Appendix B: Plagiarism declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Kudakwashe Nyamupangedengu (Student number: 1043 152) am a student registered for the degree of PhD in the academic year 2025.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date: 18/02/2025

Appendix C: Student's contribution to articles and agreement of co-authors

Declaration: Student's contribution to articles and agreement of co-authors

I, Kudakwashe Nyamupangedengu, student number 1043152, declare that this thesis is my own work and that I contributed adequately towards research findings published in the article stated below which is included in my Thesis.

Signature of student ..... **Date** .....20 February 2025 ....

Name of primary supervisor.....Prof Michelè Ramsay.....

Signature of primary supervisor ..... **Date** .....

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article by the student as part of his thesis.

Article 1

Title: Hypertension treatment practices and outcomes at health community centres in urban Soweto, South Africa

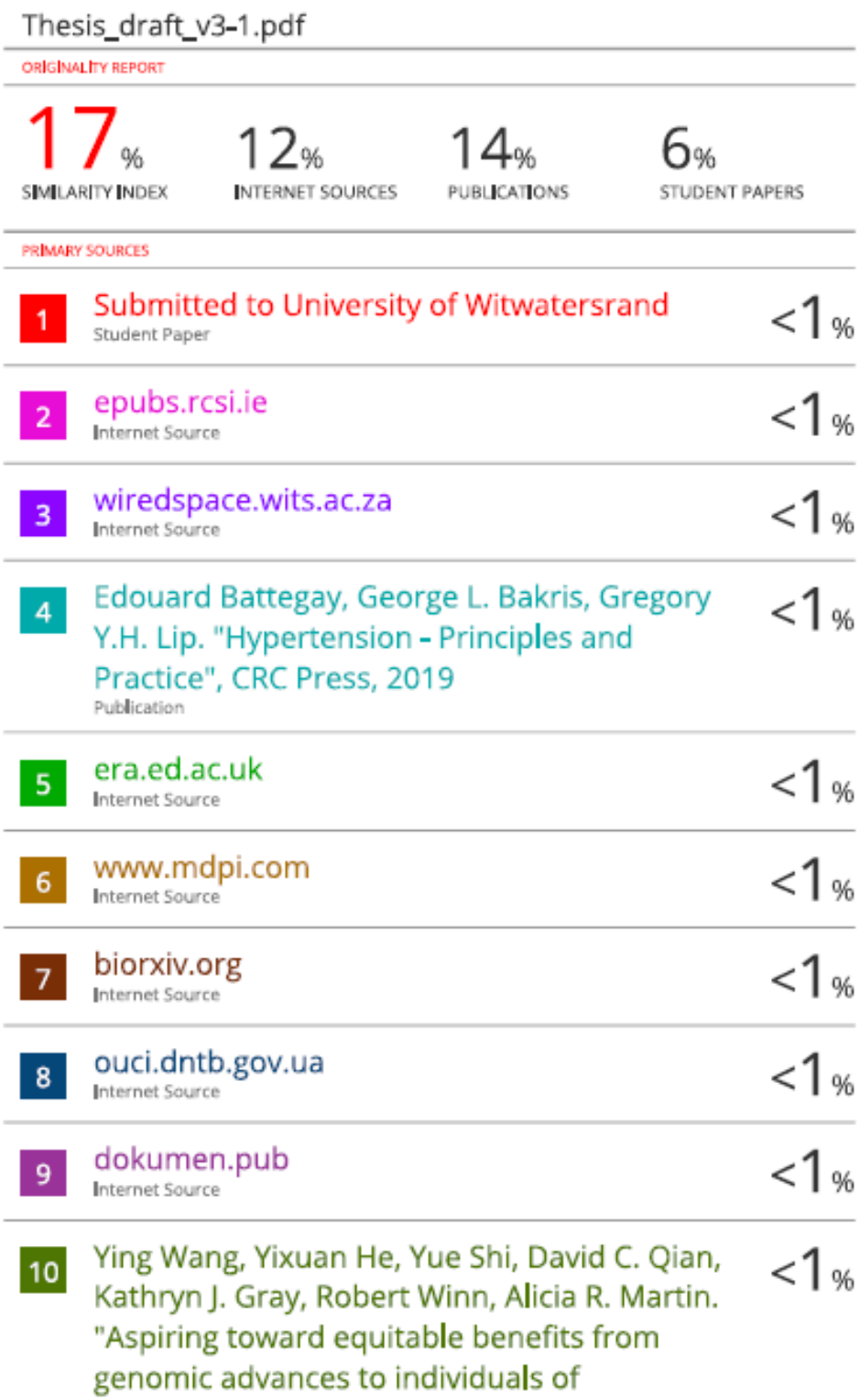
Journal name, year: *Cardiovascular Journal of Africa*, 2024.

Authors	Name	Signature	Date
1st Author	Kudakwashe Nyamupangedengu		20/02/2026
2nd Author	David Twesigomwe		26 Feb 2025
3rd Author	Andrew May		27 February 2025
4th Author	Neil Martinson		21 Feb 2025
5th Author	Ziyaad Waja		20 February 2025
6th Author	Raadhiya Osman		21 Feb 2025
7th Author	Collen Masimirembwa		
8th Author	Michelè Ramsay		

Appendix D: Use of AI

ChatGPT was used as an alternative search engine to Google. ChatGPT was also used to constrict the paper abstracts to fit into the required word count.

Appendix E: Turnitin report



Thesis_draft_v3-1.pdf

ORIGINALITY REPORT

17%	12%	14%	6%
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10	Ying Wang, Yixuan He, Yue Shi, David C. Qian, Kathryn J. Gray, Robert Winn, Alicia R. Martin. "Aspiring toward equitable benefits from genomic advances to individuals of	<1%

Appendix F: Published Paper 1

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Clinical and Translational Science

ARTICLE OPEN ACCESS

Genetic Variation in Hydrochlorothiazide Response-Related Genes Among Hypertensive Individuals in Soweto, South Africa

Kudakwashe Nyamupangedengu[1,2] | David Twesigomwe[1] | Andrew May[1,3] | Collen Masimirembwa[1,4] | Neil Martinson[5] | Ziyaad Waja[5] | Matebogo Letutu[5] | Michèle Ramsay[1]

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ABSTRACT

Africa bears the largest burden of morbidity and mortality from hypertension (HT) and related cardiovascular diseases. Thiazide diuretics, including hydrochlorothiazide (HCTZ), are the most prescribed hypertension medicines globally due to their cost effectiveness and minimal side effects. However, studies investigating the genetic variation in HCTZ-related genes and their effect on drug response have been mainly conducted in European, American, and Asian populations. This study aimed to examine genetic variation in HCTZ response-related genes among newly diagnosed hypertensives from Soweto, South Africa, irrespective of the treatment approach, and to assess its potential impact on medication response. Using targeted NGS on 364 newly diagnosed hypertensives with at least one follow-up visit, we identified 1258 unique variants in 16 key HCTZ response genes. Among these, 89 were novel variants, of which 12 were protein-altering, including 8 predicted to be deleterious. Association analyses with 882 independent variants revealed that carriers of the intronic variant rs1025450259 in *GNB3* had significantly greater reduction in BP compared to other patients on heterogeneous treatment strategies, but no significant associations were detected in the subset of individuals treated with HCTZ monotherapy only ($n = 103$). For variants previously associated with HCTZ response, we did not replicate prior associations (e.g., rs2611316 in *ALDH1A2*) but highlight important allele frequency differences across global populations. Despite the limitations, the study outcomes support the need for larger, controlled trials to assess genetic influences on HT treatment outcomes, advancing personalized medicine for African populations. This research further underscores the importance of pharmacogenetic studies in continental African populations.

1 | Introduction

Cardiovascular diseases (CVDs) are among the leading causes of morbidity and mortality globally [1]. In the sub-Saharan Africa (SSA) region, the burden of CVDs is increasing. In 2021, CVDs were reported as the second leading cause of all deaths in SSA (11%) [2], with hypertension (HT) the leading risk factor [3]. In South Africa, the burden of HT is alarmingly high and contributes substantially to cardiovascular disease mortality [4, 5]. Improving HT management and treatment outcomes, therefore, remains a public health priority for South Africa and the African continent.

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Summary

- What is the current knowledge on the topic?
  - There are population differences in responses to hypertension treatment, and genetic variants have been associated with this differential response, including to the thiazide diuretic, hydrochlorothiazide.
- What question did this study address?
  - Whether there is genetic variation in hydrochlorothiazide-related genes in the South African population and how it could impact response to treatment.
- What does this study add to our knowledge?
  - Eight variants previously associated with hydrochlorothiazide response in European, American, and Asian populations were not significantly associated in our study. Novel variants, some potentially impacting the protein product (as predicted by in silico tools) were identified in this study, thus underscoring the rich genetic diversity in continental African populations in hydrochlorothiazide-related genes.
- How might this change clinical pharmacology or translational science?
  - The critical, yet underexplored, population-specific genetic diversity potentially plays a role in drug response and hypertension management. This study emphasizes the need for continued investment in genetic studies and the development of continental African-appropriate prognostic tools as an essential approach for advancing personalized medicine through better understanding the role of genetics in creating more equitable and effective hypertension treatments and practices.

Several evidence-based treatment guidelines have been published for the management of elevated blood pressure and HT [6, 7]. BP control is a critical outcome for HT management, directly impacting the risk of death and other CVD-related complications [8]. HT management guidelines often contain specific recommendations for individuals with African ancestry [7] who have a higher prevalence of low renin salt-sensitive hypertension and adverse cardiac outcomes compared to individuals of European ancestry [9]. Furthermore, international clinical trials have shown population-specific differences in BP response to antihypertensive drugs [10, 11]. Diuretics and calcium channel blockers have been found to elicit a better BP response in African Americans compared to European Americans [10]. Similarly, in sub-Saharan African populations, dual therapy with diuretics and calcium channel blockers to be more effective compared to dual therapy with ACE inhibitors and [12]. As a result, HT management guidelines recommend treatment with either diuretics or calcium channel blockers as first-line therapy for individuals with African ancestry [6–8].

Studies have also shown that diuretics are the most cost effective first-line strategy for treating hypertension in many countries [13, 14]. Among diuretics, hydrochlorothiazide (HCTZ; a thiazide diuretic) is the leading prescribed medication, alone or in combination, in sub-Saharan Africa [3, 15]. HCTZ inhibits sodium reabsorption in the distal convoluted tubules of the kidney [16] and is particularly beneficial as part of combination therapy as it blunts water/sodium retention effects of other antihypertension drug classes [17].

According to genome-wide association studies (GWAS) on large clinical trial participants, the interindividual response to HCTZ and other antihypertensives is influenced by genetics [18]. For example, variants in the HMG-CoA synthase gene (*HMGCS*) have been associated with adverse reactions to hydrochlorothiazide (HCTZ) in African and European Americans [19]. Furthermore, variation in important BP-regulating genes *ACE, ADD1*, and *NEDD4L* has been associated with varied responses to HCTZ in multiple populations [20, 21]. Understanding the contribution of genetics in HCTZ response is therefore particularly important for diverse biogeographical groups, such as those in SSA, which rely heavily on the drug for managing hypertension.

The role of genetic variation in antihypertensive drug response in continental African populations is not well understood, as genetic studies in these populations are scarce [22]. Addressing this gap is an important step in optimizing treatment efficacy, preventing adverse reactions, and maximizing cost-effectiveness in hypertension management in Africa. The aims of the study were: (1) To examine genetic variation in 16 HCTZ response-related genes in newly diagnosed hypertensives in Soweto, South Africa, irrespective of treatment approach; (2) To investigate the potential impact of genetic variation on response to treatment, particularly HCTZ as monotherapy and combination therapy. We hypothesized that, in addition to known variants, unique genetic variations within continental African populations contribute to the efficacy of HCTZ, influencing HT management.

2 | Materials and Methods

2.1 | Study Participants

This study was approved by the University of Witwatersrand, Human Research Ethics Committee (Medical) (reference numbers: M220286 and M201143). Participants were recruited from community health clinics in Soweto, South Africa. Soweto is a historically significant township with a large, eclectic, and socioeconomically diverse population [23]. The healthcare system of Soweto is served by numerous public clinics but struggles with high patient volumes, limited resources, infrastructure constraints, and staff shortages. Informed consent was obtained from all participants prior to enrollment in the study. Inclusion criteria were adults, 18 years or older, newly diagnosed; who had been diagnosed with hypertension (SBP > 140 and/or DBP > 90) but had not taken anti-hypertensive medication in the 3 months prior to enrollment; were not taking other medications known to affect hypertension prior to enrollment; and were nonpregnant. To be included in this study, the participants were required to return for at least one study follow-up visit at which BP was measured. At baseline, study nurses recorded anthropometric and blood pressure measurements and obtained a venous blood sample (collected in the anticoagulant, ethylenediaminetetraacetic acid) for genetic analyses. For blood pressure,

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three measurements were taken using an Omron M6 (Omron Healthcare, Kyoto, Japan) at 2 min intervals. The first measurement was discarded, and the remaining two measurements were averaged and used in the study. Participants were given a follow-up date between 10 and 14 days after their baseline visit and telephonically encouraged to return; however, many returned outside of the recommended follow-up window. At follow-up visit(s), anthropometric and blood pressure measurements were re-recorded along with treatment adherence (self-reported) and if there had been any alterations to the original prescription for HT medication at baseline. Blood pressure control, that is positive response to treatment, was determined as SBP < 140 and DBP < 90. A responder was defined as having controlled BP at their study follow-up. On the basis of estimations using Quanto [24], we initially targeted a sample size of 200 responders and 200 nonresponders, which would have afforded 80% power to detect rare variants (MAF < 0.05) with an odds ratio effect size of 1.5, assuming a significance level of 0.05. The odds ratio benchmark was selected based on the typically large effect sizes of pharmacogenetic variants compared to variants associated with dichotomous complex traits including HT [25, 27].

2.2 | Sample Preparation

Genomic DNA was extracted from blood buffy coat samples using the Chemagic360 (PerkinElmer Inc., Waltham, Massachusetts). DNA quality and quantity were assessed using spectrophotometry. DNA was quantified on the Qubit 3 fluorometer using the Qubit dsDNA broad range assay kit (Invitrogen, Waltham, Massachusetts). The AmpliSeq Library PLUS kit (Illumina Inc., San Diego, California) was used to prepare the sequencing libraries according to the manufacturer's instructions. Target regions of candidate genes were amplified using primer pools, as part of the library preparation process.

2.3 | Candidate Gene Selection and Panel Design

Candidate genes, namely *ADD1, RAD52, WNK1, NEDD4L, ACE, AGTR1, YEATS, NOS3, PRKCA, GNB3, ALDH1A2, TXNDC11, TTC6, SCNN1G, HSD31B*, and *ADO*, were selected based on a prioritization approach of previous evidence of a role in HCTZ drug response in PharmGKB (described in the Supporting Information S1) [26]. A custom targeted gene panel was designed using the DesignStudio tool (Illumina Inc., San Diego, California). The primers were designed to target the exons, exon-intron boundaries, 5′ and 3′ untranslated regions, and regulatory regions of the candidate genes aiming for amplicons of approximately 150 bp in length (Table S1). The total size of the panel of 737 amplicons for 16 genes was 103,973 bp, and the probes covered 99.1% of the target regions.

2.4 | High-Coverage Targeted Sequencing and Variant Analysis

The Illumina iSeq 100 (Illumina Inc., San Diego, California), using 2 × 150 bp paired-end chemistry, was used to sequence libraries of pooled amplicons loaded onto a standard flowcell, according to the manufacturer's instructions. Sequencing performance was monitored using a PhiX (1%) positive control. The Illumina sequencing analysis viewer was used to assess sequencing quality. Raw sequencing reads were mapped to the human genome build GRCh38/hg38 using Burrow-Wheeler Aligner (BWA-MEM) [28]. Off-target intergenic variants were excluded. Tools from the Genome Analysis Toolkit (GATK), including HaplotypeCaller, GenomicsDBImport, GenotypeGVCFs, and SelectVariants [29], were applied for SNP and INDEL joint-calling for all samples [30, 31]. The full workflow used and parameters are available online (https://github.com/Kudashian/PGxHT). For variant filtration, we excluded variants with quality-by-depth (QD) < 2.0, Fisher strand (FS > 60.0), Strand Odds Ratio (SOR > 3.0), mapping quality (MQ < 40.0), mapping quality rank sum test (MQRankSum < −12.5), and read position rank sum test (ReadPosRankSum < −8.0).

2.5 | Variant Annotation and Predictions

Variants were annotated and classified using the Ensembl Variant Effect Predictor (VEP) web interface [32]. Scores from the Combined Annotation Dependent Depletion (CADD) [33] tool were used to assess the potential effect of all variants identified in the study. CADD integrates multiple annotations into a single metric, thus capturing diverse information relevant to variant effect, as opposed to tools that rely on information from a single information type (e.g., conservation). Scores from the Rare Exome Variant Ensemble Learner (REVEL) tool were used as additional evidence to predict the impact of missense variants. REVEL, a meta-predictor, predicts deleteriousness based on 13 other prediction tools [34]. SpliceAI [35], SIFT Indel [36], and LOFTEE [37] were additionally used for predicting the impact of splice, insertion/deletion, and frameshift variants respectively. All variants that were predicted to be deleterious by either CADD (score ≥ 15) or REVEL (≥ 0.4) were considered potentially impactful to the protein function. For SpliceAI, SIFT Indel, and LOFTEE, we used the "benign" and "loss-of-function" output labels for the variants.

2.6 | Statistical Analysis

We conducted a total of four association analyses using Plink v.1.9 (https://www.cog-genomics.org/plink/1.9/) [38]:

i. A logistic regression to investigate the association between genetic variants and treatment outcomes among all participants, irrespective of treatment approach. The cohort was stratified as responders (controls) or nonresponders (cases) based on whether they had attained blood pressure control at follow-up (systolic and diastolic blood pressure equal to or below 140/90).

ii. A linear regression to investigate the association between genetic variants and reduction in systolic and diastolic blood pressure from baseline to follow-up.

iii. A logistic regression to investigate the association between genetic variants and treatment outcomes in the subgroup of individuals treated only with HCTZ monotherapy. This subset of the cohort was stratified as responders (controls) or nonresponders (cases) based on whether they had

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attained blood pressure control at follow-up (systolic and diastolic blood pressure equal to or below 140/90).

iv. A linear regression to investigate the association between genetic variants and the reduction in systolic and diastolic blood pressure in the subgroup of individuals treated only with HCTZ.

Association analyses iii and iv were conducted because our candidate genes were chosen based on prior knowledge of association to HCTZ treatment outcomes. Age, sex, and BMI were used as covariates in all regression models. All data wrangling and visualizations were done in R (v 4.3.2) and RStudio (v 2023.6.0.42) using the tidyverse (v 2.0.0) and smartsnp (v 1.1.0) packages.

3 | Results

3.1 | Study Participants

A total of 556 participants (Figure 1) with hypertension were enrolled in the study and data was collected on the treatment prescribed by the treating clinician. Of these, 364 (65%) returned for at least one follow-up visit and were eligible and included in this genetic study. Drug classes prescribed to these participants included diuretic (HCTZ), calcium channel blockers (Amlodipine and Nifedipine), ACE inhibitor (Enalapril) and α-blockers (Cardura and Methyldopa) and are detailed in Table S2. Combination therapy with thiazide diuretic and calcium channel blocker (41% of participants) was the most common treatment strategy for this cohort (Table S2). For participants treated with a single drug ($n = 146$), 71% were given a thiazide diuretic (Table S2). About half of the participants in the study (185/364) reached blood pressure control in response to treatment at first follow-up. For the sub-group of participants treated with HCTZ monotherapy ($n = 103$), 47/103 (46%) had controlled BP at first follow-up. The proportion of responders was higher in females compared to males (Table 1). Baseline characteristics including BMI, waist circumference, age and sex, and average systolic and diastolic blood pressure were similar between responders and nonresponders (Table 1). The average number of days between the baseline and follow-up visits was 114 days for the cohort. This period was significantly higher in the participants with controlled BP at follow-up compared to those with uncontrolled BP; however, there was no significant difference in follow-up periods between males and females, across clinics, and between the treatment subgroups (Table S5 and Figure S2). The blood pressure response is further detailed in the (Figure S3, Tables S6 and S7).

3.2 | Variant Discovery, Annotation, and Prediction

The amplicons from the 364 samples were sequenced over 8 runs, and each run had a Phred quality score (Q30) value above 90% (for each run, 90% of the reads were sequenced with an error rate of 1/1000). The mean sequencing depth for the panel was 172× (SD = 15.77) and the mean sequencing depth for amplicons in each gene was above 100× reads (Figure S1). A total of 1258 unique variants were called, of which 89 (7% of total variants) were novel (i.e., not reported in dbSNP). The majority ($n = 603$; 48%, 603/1258) of variants in the call set were classified as intronic variants (Figure 2), and variants in coding regions ($n = 188$) comprised 15% (188/1258) of the call set. Of the coding region variants, 51% (96/188) were synonymous, and 45% (85/188) were missense variants, and only 4% (7/188) were either stop gain, indel, or frameshift (Figure 2).

The scores from prediction tools for potentially protein-altering variants (12 novel and 80 known, i.e., with an already assigned

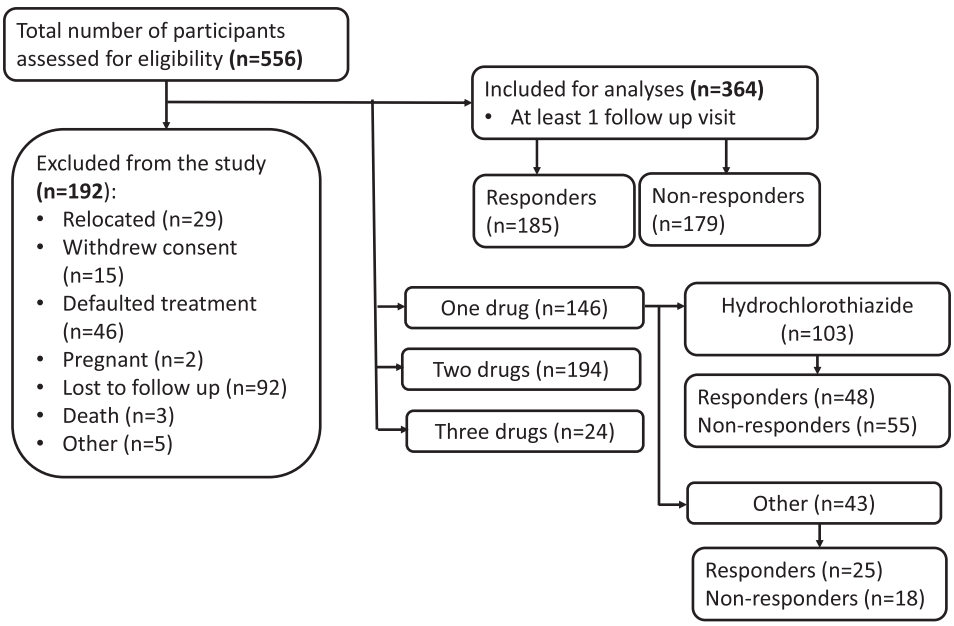


FIGURE 1 | Flow of participants recruited into the study and stratified according to groups of interest: Overall responders and nonresponders, and responders and nonresponders in individuals treated with HCTZ monotherapy. Responders, participants with BP (SBP/DBP) below 140/90 mmHg.

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TABLE 1 | Baseline characteristics of patients, stratified as responders and nonresponders to treatment at first follow-up visit.

Variables	Overall ($N=364$)	Responders ($N=185$)	Nonresponders ($N=179$)	p[c]
Age (years)[a]	49 (11)	50 (11)	48 (12)	0.059
Sex[b]				
Female	217 (60%)	126 (68%)	91 (51%)	<0.001
Male	147 (40%)	59 (32%)	88 (49%)	
BMI[a]	30 (7)	30 (7)	30 (8)	0.5
Lifestyle factors[b]				
Active smokers	72 (19%)	33 (18%)	39 (22%)	0.3
Alcohol consumers	164 (45%)	74 (40%)	90 (50%)	0.049
Comorbidities[b]				
Diabetes	14 (4%)	9 (5%)	5 (3%)	0.4
Stroke	0 (0)	0 (0)	0 (0)	N/A
Tuberculosis	7 (2%)	4 (2%)	3 (2%)	>0.9
Blood pressure (mmHg)[a]				
Systolic	157 (21)	154 (21)	161 (21)	<0.001
Diastolic	97 (14)	94 (14)	100 (14)	<0.001
Pulse rate (bpm)[c]	78 (68.5, 88.13)	76 (65, 87)	80 (71, 90)	0.002
Waist circumference (cm)[a]	93 (16)	93 (16)	93 (16)	>0.9
Number of drugs at baseline[b]				
One drug	146 (40%)	73 (39%)	73 (41%)	
Two drugs	194 (53%)	101 (55%)	93 (52%)	0.8
Three or more drugs	24 (7%)	11 (6%)	13 (7%)	
Days between visits (days)[d]	67 (27,203)	82 (31,210)	49 (18,143)	0.008

[a]Mean (SD).
[b]n (%).
[c]Median (IQR).
[d]Pearson's Chi-squared test; Fisher's exact test; Wilcoxon's rank sum test.

reference ID) indicated that almost half (40/80) of the known variants were predicted to be deleterious by at least one tool (Table 2). For the novel coding region variants (Table 3), 67% (8/12) were predicted to be deleterious by at least one tool.

Only three variants had a consensus deleterious prediction (multiple tools predicting a variant to be deleterious). Missense variant rs2049695991 (*NEDD4L*) and chr4:2898251~A>G (a novel missense variant in *ADD1*) were predicted to be deleterious by both CADD and REVEL, although they were rarely seen in the cohort, with an allele frequency less than 1%. The rs2049695991 variant had a CADD score of 32 and a REVEL score of 0.71, and the novel *ADD1* variant had a CADD score of 25.4 and a REVEL score of 0.61. For rs746484082, a multi-allelic frameshift mutation in *WNK1*, each allele was predicted to be deleterious by both CADD and LOFTEE. All splice variants were predicted to be neutral by both CADD and SpliceAI (Table S4). Over half of the missense variants (44/85) were predicted to be deleterious by CADD only. The only stop gain variant in *ACE* (COSV52012081) was predicted to be deleterious by CADD.

We identified the presence of 8 (rs10995311, rs5443, rs5723, rs5729, rs4149601, rs1008899, rs5186 and rs1799983) variants in our dataset, that had been previously reported to have an association with HCTZ response in European, American and Asian populations. Variants rs10995311 (missense variant in *ADO*) and rs5186 (3′UTR variant in *AGTR1*) were rare in our cohort but common in European and Asian populations according to the 1000 Genomes database (Table 4).

3.3 | Variant Association

Linkage disequilibrium pruning (multiple correlation coefficient over 0.5) removed 376 variants from the call set, leaving 882 independent variants (Figure S4). In association analyses (i) and (ii), we included all 364 participants (185 controls/responders and 179 cases/nonresponders) that had returned for at least one follow-up, regardless of treatment regimen. In association analysis (i), none of the 882 variants were significantly associated with blood pressure control at first follow-up, after correction for multiple testing

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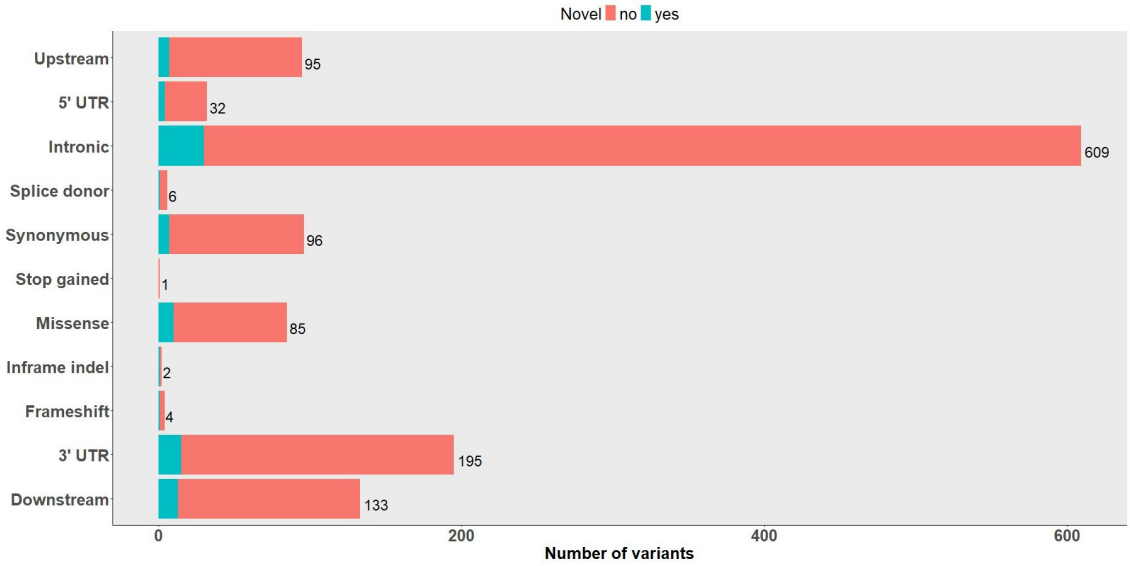


FIGURE 2 | Classification of variants ($n = 1258$) called in the cohort according to Ensembl VEP using the location of the variant. Variants in coding regions further classified by potential consequence. Novel and known variants are distinguished by blue and red shading respectively.

(p-value $< 5.67 \times 10^{-5}$). In association analysis (ii), a 3′UTR variant in *GNB3* (rs1025450259) was significantly associated with change in systolic ($p < 0.001$ after correction) and diastolic ($p < 0.001$ after correction) blood pressure, between baseline and follow-up (Table S3). For association analyses (iii) and (iv), we focused on individuals treated with HCTZ monotherapy only ($n = 103$; 48 controls/responders and 55 cases/nonresponders). None of the independent variants ($n = 882$) were significantly associated with blood pressure control at first follow-up, after correction for multiple testing, or with change in either systolic or diastolic blood pressure at first follow-up. Importantly, post hoc power analysis using our final sample size of 179 cases and 185 controls, and the Bonferroni-corrected significance threshold, suggested we were less than 27% powered to detect variants with an effect size of 1.5.

4 | Discussion

This study provides insights into genetic variation in known HCTZ response-related genes in a cohort of treatment-naive hypertensives from Soweto, with a view towards exploring the influence of genetic diversity on hypertension (HT) management in a South African context. HT management is challenging due to its multifactorial nature, influenced by genetic, environmental, and physiological factors. High-income countries report HT control rates of 43% for women and 37% for men, while sub-Saharan Africa and Oceania have the lowest HT control rates globally [40]. This disparity is likely influenced by factors such as healthcare accessibility [41, 42], the burden of comorbidities, urbanization [43, 44], and socioeconomic disparities [45, 46]. Despite these challenges also being present in South Africa, we observed that 51% of participants (58% for women and 40% for men) in a sub-set of clinics in Soweto achieved control of their HT at the first follow-up visit. However, there are significant knowledge gaps regarding the contribution of genetic variation towards the variability in response to commonly prescribed antihypertensives. Understanding how genetic variation affects HT treatment response is important for developing population-specific pharmacotherapy, particularly for continental African populations, which are underrepresented in genetic studies.

Despite the immense genetic diversity in continental African populations, they account for only 0.19% of participants in global genome-wide association studies (GWAS) [47]. Genetic diversity in Africa, characterized by low linkage disequilibrium (LD) and high complex population structure [48–50], presents unique challenges and opportunities for pharmacogenetics research. Using targeted next-generation sequencing and a custom panel, we were able to discover a considerable number of novel variants ($n = 89$; 7% of the total number of identified variants) in genes linked to HCTZ response. Twelve of these variants are of interest as they are situated in protein-altering regions and potentially affect function. This was supported by in silico tools flagging 8 variants as having a high probability to be deleterious. These findings highlight the need to explore genetic variation and its effect on drug response in continental Africans.

We observed differences in population allele frequencies of key variants previously reported to have an association with HCTZ response. Particularly, rs10995311 (*ADO*) and rs5186 (*AGTR1*) are common in European and Asian populations, but rare in our cohort, suggesting possible population-specific differences in genetic contributions to HT medication response. While several studies have examined genetic variation in HCTZ response (with the outcomes of systolic and diastolic BP reduction), we have found only three studies conducted in America, South Africa, and Brazil where blood pressure (BP) control was used as an outcome. In the American study, rs2611316 (in *ALDH1A2*) was identified (and replicated) and found to be associated with increased risk of uncontrolled BP [51]. However, this variant was not observed in our cohort, and contrary to findings from other studies, we did not find associations between variants in *ACE, ADD1, ALDH1A2*, or *SCNN1G* and treatment response. In the Brazilian study, genetic polymorphisms in *ACE* were investigated and not found to influence response to HCTZ [52]. In the South African study, rs2070744 (*NOS3*) was associated with

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TABLE 2 | Frequency and functional predictions of known protein-altering variants observed in the cohort.

RSID	Gene	Classification	REVEL[a]	CADD[b]	AF (SA)[c]	AF (Africa)[d]
rs6201(A>G)	*HSD3B1*	Missense	0.21	1.682	0.376	0.39
rs6684974(G>A)	*HSD3B1*	Missense	0.25	12.9	< 0.01	< 0.01
rs587607443(A>G)	*HSD3B1*	Missense	0.25	8.284	0.011	—
rs6205(T>C)	*HSD3B1*	Missense	0.12	0.859	0.426	0.46
rs1047303(C>A)	*HSD3B1*	Missense	0.13	0.025	0.872	0.91
rs2236295(G>T)	***ADO***	**Missense**	**0.05**	**24.1**	**< 0.01**	**0.02**
rs10995311(C>G)	*ADO*	Missense	0.09	14.73	0.018	0.04
rs766866164(G>A)	***WNK1***	**Missense**	**0.05**	**17.45**	**< 0.01**	**—**
rs11554421(G>A)	***WNK1***	**Missense**	**0.09**	**15.17**	**0.029**	**0.017**
rs971232152(G>C)	*WNK1*	Missense	0.10	7.907	< 0.01	—
rs34728563(T>C)	***WNK1***	**Missense**	**0.09**	**20.8**	**0.04**	**0.05**
rs746484082(GA>G)	***WNK1***	**Frameshift**	**NA**	**30**	**0.394**	**< 0.01**
rs746484082(GA>GAA)	***WNK1***	**Frameshift**	**NA**	**30**	**0.012**	**< 0.01**
rs746484082(GA>GAAAA)	***WNK1***	**Frameshift**	**NA**	**30**	**0.014**	**< 0.01**
rs764368160(A>G)	***WNK1***	**Missense**	**0.08**	**16.85**	**0.01**	**—**
rs11833299(A>G)	***WNK1***	**Missense**	**0.15**	**24.6**	**0.021**	**0.03**
rs61736905(T>C)	*WNK1*	Missense	0.04	11.92	0.038	0.05
rs139449198(A>G)	*WNK1*	Missense	0.05	13.03	< 0.01	< 0.01
rs762367651(C>T)	***WNK1***	**Missense**	**0.08**	**17.49**	**< 0.01**	**—**
rs956868(A>C)	*WNK1*	Missense	0.05	7.934	0.834	0.86
rs138216561(A>G)	*WNK1*	Missense	0.09	7.828	< 0.01	—
rs368621387(G>A)	*WNK1*	Missense	0.08	0.243	< 0.01	—
rs575881382(C>G)	*WNK1*	Missense	0.06	7.9	< 0.01	< 0.01
rs7955371(G>C)	*WNK1*	Missense	0.12	3.922	0.938	0.95
rs146861951(G>A)	***WNK1***	**Missense**	**0.02**	**18.34**	**< 0.01**	**—**
rs748898332(G>C)	***WNK1***	**Missense**	**0.05**	**15.48**	**< 0.01**	**—**
rs12828016(G>T)	***WNK1***	**Missense**	**0.01**	**15.64**	**0.514**	**0.55**
rs61747607(A>G)	*GNB3*	Missense	0.19	0	0.019	0.01
rs748642355(G>A)	***TTC6***	**Missense**	**NA**	**16.47**	**< 0.01**	**< 0.01**
rs999476305(A>G)	***TTC6***	**Missense**	**NA**	**23.1**	**< 0.01**	**—**
rs768751920(C>T)	*TTC6*	Missense	NA	0.709	0.00137	< 0.01
rs116578389(C>T)	*TTC6*	Missense	NA	11.01	0.058	0.05
rs12896790(T>G)	*TTC6*	Missense	0.05	9.621	< 0.01	0.05
rs4901284(C>T)	***TTC6***	**Missense**	**0.07**	**16**	**0.162**	**0.29**
rs17107176(A>T)	*TTC6*	Missense	0.03	0	0.224	0.07
rs61740232(T>C)	*TTC6*	Missense	0.40	**23**	< 0.01	< 0.01
rs754819517(C>T)	***ALDH1A2***	**Missense**	**0.15**	**19.69**	**< 0.01**	**—**
rs4646626(C>T)	***ALDH1A2***	**Missense**	**0.18**	**17.37**	**0.29**	**0.38**

(Continues)

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TABLE 2 | (Continued)

RSID	Gene	Classification	REVEL[a]	CADD[b]	AF (SA)[c]	AF (Africa)[d]
rs115875978(C>A)	***ALDH1A2***	**Missense**	**0.45**	**24.9**	**0.019**	**<0.01**
rs1272956564(T>C)	*TXNDC11*	Missense	0.08	8.062	<0.01	—
rs144296098(T>A)	***TXNDC11***	**Missense**	**0.16**	**24.4**	**<0.01**	**<0.01**
rs560075677(G>C)	***TXNDC11***	**Missense**	**0.06**	**16**	**<0.01**	**0.02**
rs534277277(G>C)	***TXNDC11***	**Missense**	**0.04**	**23**	**<0.01**	**<0.01**
rs3743588(G>A)	***TXNDC11***	**Missense**	**0.03**	**22**	**0.027**	**0.02**
rs781662574(C>T)	***TXNDC11***	**Missense**	**0.05**	**22**	**0.019**	**<0.01**
rs929252570(T>C)	***TXNDC11***	**Missense**	**0.04**	**16**	**<0.01**	—
rs5736(G>A)	*SCNN1G*	Missense	0.14	1.042	0.041	0.04
COSV55597704(G>C)	***SCNN1G***	**Missense**	**0.13**	**24**	**<0.01**	—
rs532691783(T>TGCTGCC)	*ACE*	Inframe indel	NA	7.649	<0.01	—
rs4317(T>C)	*ACE*	Missense	0.01	0.924	0.276	0.2
rs4318(A>G)	*ACE*	Missense	0.01	6.912	0.257	0.2
COSV52014914(C>G)	*ACE*	Missense	0.10	12.75	<0.01	—
rs3730043(C>T)	***ACE***	**Missense**	**0.22**	**24.7**	**<0.01**	**<0.01**
rs551723440(A>G)	*ACE*	Missense	0.03	13.36	0.027	—
COSV52012081(G>A)	***ACE***	**Stop-gain**	**NA**	**48**	**<0.01**	—
COSV52006486(C>A)	***ACE***	**Missense**	**0.45**	**24.7**	**<0.01**	—
rs144751624(T>A)	*ACE*	Missense	0.46	6.743	<0.01	<0.01
rs145349565(C>A)	***ACE***	**Missense**	**0.48**	**24.1**	**<0.01**	**<0.01**
rs4980(G>A)	***ACE***	**Missense**	**0.05**	**23.2**	**<0.01**	**<0.01**
rs4364(C>A)	*ACE*	Missense	0.05	13.31	0.126	0.13
rs12720745(G>A)	*ACE*	Missense	0.06	0.004	<0.01	<0.01
rs61762396(G>A)	*PRKCA*	Missense	NA	7.915	0.038	0.01
rs141376042(G>A)	***PRKCA***	**Missense**	**0.06**	**20.8**	**0.025**	**<0.01**
rs968257095(C>T)	***NEDD4L***	**Missense**	**0.13**	**23.9**	**<0.01**	—
rs767136811(C>T)	***NEDD4L***	**Missense**	**0.03**	**24.1**	**<0.01**	—
rs2049695991(G>T)	***NEDD4L***	**Missense**	**0.71**	**32**	**<0.01**	—
rs111980524(G>A)	*AGTR1*	Missense	0.14	9.103	0.012	<0.01
rs146536048(G>A)	***ADD1***	**Missense**	**0.09**	**17.25**	**0.012**	**<0.01**
rs4963(C>G)	***ADD1***	**Missense**	**0.09**	**18.12**	**0.228**	**0.18**
rs199688227(G>A)	*NOS3*	Missense	0.02	0.331	<0.01	<0.01
rs3918166(G>A)	*NOS3*	Missense	0.05	13.33	0.04	0.02
rs149224629(A>T)	***NOS3***	**Missense**	**0.08**	**22.1**	**<0.01**	**<0.01**
COSV52496818(G>C)	*NOS3*	Missense	0.02	13.28	<0.01	—
rs377756718(G>A)	***NOS3***	**Missense**	**0.20**	**24**	**<0.01**	—
rs41377046(G>A)	***NOS3***	**Missense**	**0.24**	**22.3**	**<0.01**	—
rs1799983(T>G)	*NOS3*	Missense	0.06	10.07	0.202	0.93

(Continues)

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TABLE 2 | (Continued)

RSID	Gene	Classification	REVEL[a]	CADD[b]	AF (SA)[c]	AF (Africa)[d]
rs145168353(G>A)	***NOS3***	**Missense**	**0.08**	**22.8**	**< 0.01**	—
rs7792133(G>A)	***NOS3***	**Missense**	**0.14**	**20.8**	**< 0.01**	**0.02**
rs369652420(G>A)	***NOS3***	**Missense**	**0.08**	**19.61**	**< 0.01**	**< 0.01**
rs3918201(G>T)	***NOS3***	**Missense**	**0.10**	**22**	**0.082**	**0.05**

Note: Variants with scores denoting deleteriousness according to either CADD or REVEL formatted with bold font. All positions are according to GRCh38.
[a]REVEL deleterious criteria: score > = 0.5 = deleterious, score < 0.5 = neutral.
[b]CADD deleterious criteria: score > = 15 = deleterious, score < 15 = neutral.
[c]Allele frequency in our cohort ($n = 364$).
[d]Allele frequency according to 1000 Genomes Project, where data is available [39].

TABLE 3 | Novel protein-altering variants in the South African cohort.

Variant	Gene	Classification	Revel[a]	CADD[b]	AF[c]
chr12:754068 ~ A>T	***WNK1***	**Missense**	**0.09**	**20.2**	**< 0.01**
chr12:885344 ~ TCTA>T	*WNK1*	Inframe indel	NA	NA	< 0.01
chr14:37682826 ~ G>T	***TTC6***	**Missense**	**NA**	**20.3**	**< 0.01**
chr14:37841565 ~ A>G	*TTC6*	Missense	0.06	9	< 0.01
chr16:11688344 ~ C>T	***TXNDC11***	**Missense**	**0.37**	**25.6**	**< 0.01**
chr16:11691304 ~ A>C	*TXNDC11*	Missense	0.04	14.61	< 0.01
chr16:11736066 ~ A>T	***TXNDC11***	**Missense**	**0.24**	**28**	**< 0.01**
chr17:63497257 ~ T>C	***ACE***	**Missense**	**0.07**	**23.6**	**< 0.01**
chr3:148741747 ~ AT>A	*AGTR1*	Frameshift	NA	NA	< 0.01
chr4:2898251 ~ A>G	***ADD1***	**Missense**	**0.61**	**25.4**	**< 0.01**
chr4:2914981 ~ C>A	***ADD1***	**Missense**	**0.25**	**25.3**	**< 0.01**
chr7:150993821 ~ C>G	***NOS3***	**Missense**	**0.22**	**18.12**	**0.032**

Note: Variants with scores denoting deleteriousness according to either CADD or REVEL are indicated in bold. All positions are according to GRCh38.
[a]REVEL deleterious criteria: score > = 0.5 = deleterious, score < 0.5 = neutral.
[b]CADD deleterious criteria: score > = 15 = deleterious, score < 15 = neutral.
[c]Allele frequency ($n = 364$).

TABLE 4 | Distribution of variants with previous reported associations with HCTZ response.

RSID	Gene	Variant consequence	AF (SA)[a]	AF (1KG_Afr)[b]	AF (1KG_Eur)[b]	AF (1KG_Eas)[bs]
rs10995311(C>G)	*ADO*	Missense	0.02	0.04	0.42	0.17
rs5443(C>T)	*GNB3*	Synonymous	0.80	0.82	0.31	0.51
rs5723(C>G)	*SCNN1G*	Synonymous	0.28	0.25	0.22	0.06
rs5729(T>A)	*SCNN1G*	3′UTR	0.28	0.25	0.22	0.06
rs4149601(G>A)	*NEDD4L*	Intron	0.45	0.34	0.37	0.21
rs1008899(G>A)	*NEDD4L*	Intron	0.06	0.06	0.23	0.51
rs5186(A>C)	*AGTR1*	3′UTR	< 0.01	0.02	0.28	0.06
rs1799983(T>G)	*NOS3*	Missense	0.20	0.92	0.65	0.87

Note: All positions are according to GRCh38.
Abbreviations: Afr, African ancestry; Eas, East Asian ancestry [39]; Eur, European ancestry.
[a]AF, Allele frequency; SA, this study of South Africans ($n = 364$).
[b]Population allele frequency according to 1000 Genomes Project (1KG) (30× data).

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uncontrolled hypertension [53]. We identified an intronic variant in *GNB3* (rs1025450259) associated with a reduction in both systolic (Beta = −86.84; SE = 23.5) and diastolic (Beta = −53.97; SE = 10.15) BP. *GNB3* plays a critical role in BP regulation through its GTPase activity [54], making it a gene of interest for further study. The association of rs1025450259 with BP response to HCTZ has not been previously reported; however, the low allele frequency of rs1025450259 (MAF = 0.27%) and our limited power to detect true positives complicate further interpretation. Given the complexity of BP regulation (driven by multiple genetic, environmental, and lifestyle factors), it is difficult to pinpoint single or high-effect genetic variants that may impact treatment outcomes.

Our study emphasizes the limitations of current in silico prediction tools, which often disagreed on the consequences of protein-altering variants. The existing predictive tools yield inconclusive results for these continental African population-derived variants, as seen in our study. Continental African populations are generally underrepresented in databases that variant effect predictive tools use for training prediction algorithms. This may lead to effect predictions which are biased towards represented populations. This challenge of predictive algorithms for treatment outcomes is exacerbated by the high genetic diversity present in continental African populations, which influences the genetic architecture of specific traits [55]. Tools which are calibrated to the diverse genetic landscape of continental African populations may produce more accurate and insightful predictions.

Although all variants met filtering criteria, and the allele depths of singletons were manually inspected on Integrative Genomics Viewer (IGV) [56], the validation of novel variants identified was beyond the scope of the study. We provided in silico impact prediction scores as evidence, but technical validation (through Sanger sequencing or replication studies), functional validation (through in vitro and in vivo studies) and clinical validation are still required to fully understand the impact of the novel variants.

Our candidate gene next generation sequencing approach successfully identified novel variants and highlighted the high genetic diversity of continental African populations. However, given the infrastructural challenges of sample recruitment in a low-resource setting, we were underpowered to identify associated effects for discovered variants. In addition, the observational nature of the study prevented causal inference, and there were many additional covariates we were unable to control for (e.g., lifestyle factors). A more controlled, larger-scale study with multiple follow-ups and better monitoring of compliance and lifestyle factors would provide a more robust dataset for investigating the role of genetic variation in HT treatment outcomes among continental African hypertensives.

This study provides evidence that there is unidentified genetic variation in continental African populations in HCTZ-response related genes. Furthermore, there are potential contributions from genetic variants to BP reduction in response to treatment in this population. The lack of replication of previously identified associations with HCTZ response, as well as allele frequency differences of these variants, suggests that findings from other populations may not always be transferrable to African populations. Substantial investment and prioritization of genetic studies in continental African populations is necessary for unlocking the possibility of precision medicine as a strategy for effective HT management on the continent.

Author Contributions

M.R., N.M., A.M., Z.W., and C.M. designed the research. K.N. and M.L. performed the research. K.N. and D.T. analyzed the data. K.N., D.T., A.M., M.R., and C.M. wrote the manuscript. All authors read and agreed to the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors on request due to privacy restrictions.

References

1. WHO, "Cardiovascular Diseases (CVDs)," (2021).

2. Global Burden of Disease Collaborative Network, *Global Burden of Disease Study 2021 (GBD 2021)* (Institute for Health Metrics and Evaluation (IHME), 2024).

3. A. Seeley, J. Prynn, R. Perera, R. Street, D. Davis, and A. O. Etyang, "Pharmacotherapy for Hypertension in Sub-Saharan Africa: A Systematic Review and Network Meta-Analysis," *BMC Medicine* 18, no. 1 (2020): 75.

4. F. X. Gómez-Olivé, S. A. Ali, F. Made, et al., "Regional and Sex Differences in the Prevalence and Awareness of Hypertension: An H3Africa AWI-Gen Study Across 6 Sites in Sub-Saharan Africa," *Global Heart* 12, no. 2 (2017): 81–90.

5. N. Abdelatif, N. Peer, and S. O. Manda, "National Prevalence of Coronary Heart Disease and Stroke in South Africa From 1990–2017: A Systematic Review and Meta-Analysis," *Cardiovascular Journal of Africa* 32, no. 3 (2021): 156–160.

6. Y. K. Seedat, B. L. Rayner, and Y. Veriava, "South African Hypertension Practice Guideline 2014," *Cardiovascular Journal of Africa* 25, no. 6 (2014): 288–294.

7. T. Unger, C. Borghi, F. Charchar, et al., "2020 International Society of Hypertension Global Hypertension Practice Guidelines," *Hypertension* 75, no. 6 (2020): 1334–1357.

8. WHO, *Guideline for the Pharmacological Treatment of Hypertension in Adults* (Geneva: World Health Organization, 2021).

9. L. Faconti, R. J. McNally, B. Farukh, et al., "Differences in Hypertension Phenotypes Between Africans and Europeans: Role of Environment," *Journal of Hypertension* 38, no. 7 (2020): 1278–1285.

10. J. A. Johnson, "Ethnic Differences in Cardiovascular Drug Response: Potential Contribution of Pharmacogenetics," *Circulation* 118, no. 13 (2008): 1383–1393.

11. D. T. Lackland, "Racial Differences in Hypertension: Implications for High Blood Pressure Management," *American Journal of the Medical Sciences* 348, no. 2 (2014): 135–138.

17528662, 2025, 7, Downloaded from https://ascpt.onlinelibrary.wiley.com/doi/10.1111/cts.70302 by South African Medical Research, Wiley Online Library on [27/09/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

12. D. B. Ojji, B. Mayosi, V. Francis, et al., "Comparison of Dual Therapies for Lowering Blood Pressure in Black Africans," *New England Journal of Medicine* 380, no. 25 (2019): 2429–2439.

13. C. Park, G. Wang, B. P. Ng, J. Fang, J. M. Durthaler, and C. Ayala, "The Uses and Expenses of Antihypertensive Medications Among Hypertensive Adults," *Research in Social & Administrative Pharmacy: RSAP* 16, no. 2 (2020): 183–189.

14. M. Kučan, I. Lulić, J. M. Pelčić, V. Mozetič, and D. Vitezić, "Cost Effectiveness of Antihypertensive Drugs and Treatment Guidelines," *European Journal of Clinical Pharmacology* 77, no. 11 (2021): 1665–1672.

15. P. Cavagna, C. Leplay, R. N'Guetta, et al., "Hypertension Treatment in Sub-Saharan Africa: A Systematic Review," *Cardiovascular Journal of Africa* 34, no. 5 (2023): 307–317.

16. L. L. Herman, P. Weber, and K. Bashir, "Hydrochlorothiazide," in *StatPearls* (StatPearls Publishing, 2024).

17. N. S. Skolnik, J. D. Beck, and M. Clark, "Combination Antihypertensive Drugs: Recommendations for Use," *American Family Physician* 61, no. 10 (2000): 3049–3056.

18. J. Rysz, B. Franczyk, M. Rysz-Górzyńska, and A. Gluba-Brzózka, "Pharmacogenomics of Hypertension Treatment," *International Journal of Molecular Sciences* 21, no. 13 (2020): 4709.

19. S. Singh, C. W. McDonough, Y. Gong, et al., "Genome Wide Association Study Identifies the HMGCS2 Locus to Be Associated With Chlorthalidone Induced Glucose Increase in Hypertensive Patients," *Journal of the American Heart Association* 7, no. 6 (2018): e007339.

20. M. T. Sciarrone, P. Stella, C. Barlassina, et al., "ACE and α-Adducin Polymorphism as Markers of Individual Response to Diuretic Therapy," *Hypertension* 41, no. 3 I (2003): 398–403.

21. P. Svensson-Färbom, B. Wahlstrand, P. Almgren, et al., "A Functional Variant of the NEDD4L Gene Is Associated With Beneficial Treatment Response With β-Blockers and Diuretics in Hypertensive Patients," *Journal of Hypertension* 29, no. 2 (2011): 388–395.

22. F. Radouani, L. Zass, Y. Hamdi, et al., "A Review of Clinical Pharmacogenetics Studies in African Populations," *Personalized Medicine* 17, no. 2 (2020): 155–170.

23. P. L. Bonner and L. Segal, *Soweto: A History* (Maskew Miller Longman, 1998).

24. W. J. Gauderman, "Sample Size Requirements for Matched Case-Control Studies of Gene-Environment Interaction," *Statistics in Medicine* 21, no. 1 (2002): 35–50.

25. J. C. Maranville and N. J. Cox, "Pharmacogenomic Variants Have Larger Effect Sizes Than Genetic Variants Associated With Other Dichotomous Complex Traits," *Pharmacogenomics Journal* 16, no. 4 (2016): 388–392.

26. M. Whirl-Carrillo, R. Huddart, L. Gong, et al., "An Evidence-Based Framework for Evaluating Pharmacogenomics Knowledge for Personalized Medicine," *Clinical Pharmacology & Therapeutics* 110, no. 3 (2021): 563–572, https://doi.org/10.1002/cpt.2350.

27. R. M. Cooper-DeHoff and J. A. Johnson, "Hypertension Pharmacogenomics: In Search of Personalized Treatment Approaches," *Nature Reviews Nephrology* 12, no. 2 (2015): 110–122, https://doi.org/10.1038/nrneph.2015.176.

28. H. Li, "Aligning Sequence Reads, Clone Sequences and Assembly Contigs with BWA-MEM (Version 2)," *arXiv* (2013), https://doi.org/10.48550/ARXIV.1303.3997.

29. A. McKenna, M. Hanna, E. Banks, et al., "The Genome Analysis Toolkit: A MapReduce Framework for Analyzing Next-Generation DNA Sequencing Data," *Genome Research* 20, no. 9 (2010): 1297–1303, https://doi.org/10.1101/gr.107524.110.

30. M. A. DePristo, E. Banks, R. Poplin, et al., "A Framework for Variation Discovery and Genotyping Using Next-Generation DNA Sequencing Data," *Nature Genetics* 43, no. 5 (2011): 491–498, https://doi.org/10.1038/ng.806.

31. G. A. Van der Auwera and B. D. O'Connor, *Genomics in the Cloud: Using Docker, GATK, and WDL in Terra*, 1st ed. (O'Reilly Media, 2020).

32. W. McLaren, L. Gil, S. E. Hunt, et al., "The Ensembl Variant Effect Predictor," *Genome Biology* 17, no. 1 (2016): 122, https://doi.org/10.1186/s13059-016-0974-4.

33. M. Kircher, D. M. Witten, P. Jain, B. J. O'Roak, G. M. Cooper, and J. Shendure, "A General Framework for Estimating the Relative Pathogenicity of Human Genetic Variants," *Nature Genetics* 46, no. 3 (2014): 310–315, https://doi.org/10.1038/ng.2892.

34. N. M. Ioannidis, J. H. Rothstein, V. Pejaver, et al., "REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants," *American Journal of Human Genetics* 99, no. 4 (2016): 877–885, https://doi.org/10.1016/j.ajhg.2016.08.016.

35. K. Jaganathan, S. Kyriazopoulou Panagiotopoulou, J. F. McRae, et al., "Predicting Splicing from Primary Sequence with Deep Learning," *Cell* 176, no. 3 (2019): 535–548.e24, https://doi.org/10.1016/j.cell.2018.12.015.

36. J. Hu and P. C. Ng, "SIFT Indel: Predictions for the Functional Effects of Amino Acid Insertions/Deletions in Proteins," *PLoS One* 8, no. 10 (2013): e77940, https://doi.org/10.1371/journal.pone.0077940.

37. K. J. Karczewski, L. C. Francioli, G. Tiao, et al., "The Mutational Constraint Spectrum Quantified from Variation in 141,456 Humans," *Nature* 581, no. 7809 (2020): 434–443, https://doi.org/10.1038/s41586-020-2308-7.

38. S. Purcell, B. Neale, K. Todd-Brown, et al., "PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses," *American Journal of Human Genetics* 81, no. 3 (2007): 559–575, https://doi.org/10.1086/519795.

39. A. Auton, G. R. Abecasis, D. M. Altshuler, et al., "A Global Reference for Human Genetic Variation," *Nature* 526, no. 7571 (2015): 68–74, https://doi.org/10.1038/nature15393.

40. B. Zhou, R. M. Carrillo-Larco, G. Danaei, et al., "Worldwide Trends in Hypertension Prevalence and Progress in Treatment and Control from 1990 to 2019: A Pooled Analysis of 1201 Population-Representative Studies with 104 Million Participants," *Lancet* 398, no. 10304 (2021): 957–980, https://doi.org/10.1016/s0140-6736(21)01330-1.

41. M. Baazeem, E. Kruger, and M. Tennant, "Current Status of Tertiary Healthcare Services and Its Accessibility in Rural and Remote Australia: A Systematic Review," *Health Sciences Review* 11 (2024): 100158, https://doi.org/10.1016/j.hsr.2024.100158.

42. Z. T. Tessema, M. G. Worku, G. A. Tesema, et al., "Determinants of Accessing Healthcare in Sub-Saharan Africa: A Mixed-Effect Analysis of Recent Demographic and Health Surveys from 36 Countries," *BMJ Open* 12, no. 1 (2022): e054397, https://doi.org/10.1136/bmjopen-2021-054397.

43. A. K. McLennan and S. J. Ulijaszek, "Obesity Emergence in the Pacific Islands: Why Understanding Colonial History and Social Change Is Important," *Public Health Nutrition* 18, no. 8 (2014): 1499–1505, https://doi.org/10.1017/s136898001400175x.

44. K. Mudie, M. M. Jin, Tan, et al., "Non-Communicable Diseases in Sub-Saharan Africa: A Scoping Review of Large Cohort Studies," *Journal of Global Health* 9, no. 2 (2019): 020409, https://doi.org/10.7189/jogh.09.020409.

45. W. C. Chan, C. Wright, T. Riddell, et al., "Ethnic and Socioeconomic Disparities in the Prevalence of Cardiovascular Disease in New Zealand," *New Zealand Medical Journal* 121, no. 1285 (2008): 11–20.

46. M. Antignac, I. B. Diop, D. Macquart de Terline, et al., "Socioeconomic Status and Hypertension Control in Sub-Saharan Africa," *Hypertension* 71, no. 4 (2018): 577–584, https://doi.org/10.1161/hypertensionaha.117.10512.

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47. M. C. Mills and C. Rahal, "The GWAS Diversity Monitor Tracks Diversity by Disease in Real Time," *Nature Genetics* 52, no. 3 (2020): 242–243, https://doi.org/10.1038/s41588-020-0580-y.

48. N. Yu, F.-C. Chen, S. Ota, et al., "Larger Genetic Differences Within Africans Than Between Africans and Eurasians," *Genetics* 161, no. 1 (2002): 269–274, https://doi.org/10.1093/genetics/161.1.269.

49. M. C. Campbell and S. A. Tishkoff, "African Genetic Diversity: Implications for Human Demographic History, Modern Human Origins, and Complex Disease Mapping," *Annual Review of Genomics and Human Genetics* 9, no. 1 (2008): 403–433, https://doi.org/10.1146/annurev.genom.9.081307.164258.

50. F. Gomez, J. Hirbo, and S. A. Tishkoff, "Genetic Variation and Adaptation in Africa: Implications for Human Evolution and Disease," *Cold Spring Harbor Perspectives in Biology* 6, no. 7 (2014): a008524–a008524, https://doi.org/10.1101/cshperspect.a008524.

51. O. Magvanjav, Y. Gong, C. W. McDonough, et al., "Genetic Variants Associated With Uncontrolled Blood Pressure on Thiazide Diuretic/β-Blocker Combination Therapy in the PEAR (Pharmacogenomic Evaluation of Antihypertensive Responses) and INVEST (International Verapamil-SR Trandolapril Study) Trials," *Journal of the American Heart Association* 6, no. 11 (2017): e006522, https://doi.org/10.1161/jaha.117.006522.

52. L. da Cunha Agostini, W. de Paula, A. S. Melo, et al., "Single Nucleotide Polymorphism (SNP) rs4291 of the Angiotensin-Converting Enzyme (ACE) Gene Is Associated with the Response to Losartan Treatment in Hypertensive Patients," *Molecular Biology Reports* 51, no. 1 (2024): 458, https://doi.org/10.1007/s11033-024-09437-1.

53. C. Masilela, B. Pearce, J. J. Ongole, O. V. Adeniyi, and M. Benjeddou, "Genomic Association of Single Nucleotide Polymorphisms with Blood Pressure Response to Hydrochlorothiazide among South African Adults with Hypertension," *Journal of Personalized Medicine* 10, no. 4 (2020): 267, https://doi.org/10.3390/jpm10040267.

54. H. R. Bourne, D. A. Sanders, and F. McCormick, "The GTPase Superfamily: Conserved Structure and Molecular Mechanism," *Nature* 349, no. 6305 (1991): 117–127, https://doi.org/10.1038/349117a0.

55. M. D. Kessler, L. Yerges-Armstrong, M. A. Taub, et al., "Challenges and Disparities in the Application of Personalized Genomic Medicine to Populations with African Ancestry," *Nature Communications* 7, no. 1 (2016): 12521, https://doi.org/10.1038/ncomms12521.

56. J. T. Robinson, H. Thorvaldsdóttir, W. Winckler, et al., "Integrative Genomics Viewer," *Nature Biotechnology* 29, no. 1 (2011): 24–26, https://doi.org/10.1038/nbt.1754.

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