Inter-individual Genetic Variation and the Development of Hypertension in a Xhosa African population of Eastern Cape, South Africa

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General Abstract

Background

Cardiovascular diseases (CVD) are the leading cause of death globally, accounting for 18.6 million deaths. Hypertension (HTN) drives the global burden of CVD and is a leading cause of cardiovascular-related mortality with 1.4 billion affected adults and 10.4 million deaths globally. This public health condition has been escalating alarmingly in low and middle-income countries. In Sub-Saharan Africa, HTN is a major public health concern with South Africa having the highest prevalence between 27-58%. Accumulative evidence shows that HTN is driven by both modifiable and non-modifiable risk factors. Various non-modifiable risk factors such as genetics, have been attributed to play a significant role in the prevalence of HTN. While genetic data exist on individuals of European descent, currently, data are scarce on the indigenous South African population, particularly the role inter-individual genetic variation plays in HTN. Therefore, the aim of the study is to investigate their inter-individual genetic variation and the development of HTN in a Xhosa South African population.

Methods

To accomplish this aim, we evaluated the prevalence and associated risk factors of HTN in our study population consisting of Xhosa speaking black population in South Africa from four districts (OR Tambo, Alfred Nzo, Chris Hani, Joe Gqabi) and fourteen community health care centers in the Eastern Cape province of South Africa. Screening and diagnosis of HTN were defined as per guidelines provided by the American Heart Association (AHA), as follows: (1) Normal blood pressure (systolic blood pressure (SBP): < 120 mmHg and diastolic blood pressure (DBP) < 80mm Hg, (2) Elevated blood pressure (SBP:120–129 mm Hg and DBP < 80 mm Hg), (3) HTN grade 1 (SBP:130–139 mm Hg and /or DBP:80–89 mm Hg), and (4) HTN grade 2 (SBP ≥ 140 mm Hg and/or DBP ≥ 90. Thereafter, an *in silico* predictive approach was utilised to identify several genes and their relative SNPs including *ACE*, rs1799752; *AGT*, rs2004776; *ADRB2*, rs1042713, and *MTHFR*, rs1801133 which may act as potential drug targets for HTN and further used to perform co-expression analysis.

Blood samples were collected from each participant and DNA was extracted using the QIAamp Blood Midi Kit. SNPs previously reported to be associated with HTN in the African population such as *ACE*, rs1799752; *AGT*, rs2004776, rs3789678; *ADRB2*, rs1042713, rs1042714, and *MTHFR*, rs1801133 were genotyped using Mass Array and Validated using TaqMan SNP Genotyping Assay, and polymerase chain reaction (PCR). Minor allele frequency (MAF) and Hardy–Weinberg equilibrium (HWE) tests

were calculated using Genetic Analysis in Excel (GenAIEx) Version 6.5, where a p-value <0.05 indicated a deviation from the Hardy Weinberg principle. Furthermore, Chi-squared test with odds ratio and 95% confidence intervals (CI) was performed and the association was investigated using various inheritance models and determined by logistic regression model analysis. To enhance the study power and investigate the polymorphisms influence on the disease state, haplotype analysis was performed for linkage disequilibrium (LD). Furthermore, the interaction between selected SNPs and risk factors on HTN was performed using the open-source multifactor dimensionality reduction (MDR).

Results

The results presented in this study demonstrate that the prevalence of HTN was high (~71%) among an indigenous South African population of Mthatha town. Further, our results revealed a positive association between age, income, westernized diet, higher levels of blood glucose, BMI with the development of HTN. Additionally, it has also been demonstrated that gender, age, education, BMI, and belief in controlling HTN with medication are key predictors of determining the treatment status of HTN. This study observed that 40% of the participants were unaware of their HTN status, thus highlighting the urgent need for HTN education, screening, and control within the Mthatha area.

Furthermore, to better understand the role of inter-individual genetic variants in the development of HTN, we utilised *in silico* approach and identified 53 genes with 84 SNPs, previously been reported to be associated with HTN in Africa. Then co-expression analysis of the identified SNPs revealed three clusters, including cluster 1 (*ACE, AGT, AGTR1, AGTR2*, and *NOS3*), cluster 2 (*MOV10*, *CAN1*, and *IGF2BP2*), and cluster 3 (*CSK* and *ADRB2*). The latter is of importance, as the targeting of co-regulatory gene clusters will allow for the development of more effective HTN drug targets that could decrease the prevalence of HTN. Upon further analysis, genes such as *AGTR1*, *AGTR2*, *AGT,* and *ACE* (Cluster 1); *IGFBP2* (Cluster 2), and *ADRB2* (cluster 3) were shown to be mapped to FDA-approved HTN drugs. Interestingly, the protein-coding genes including *AGTR1*, *AGTR2, AGT,* and *ACE* all mapped back to the renin-angiotensin-aldosterone system (RAAS), which is a major regulator of blood pressure and vascular resistance. Hence, this study explored SNPs within the identified clusters (1-3) with a specific focus on SNPs within the *ACE* and *AGT* genes.

In univariate regression analysis, we observed no significant association for both *ACE* and *AGT* polymorphisms; however, multivariate analysis showed that the T allele

provides a 3-fold risk of developing HTN for rs2004776 (OR = 3.44, 95% CI = 1.070 – 11.036, p = 0.038), while rs3789678 offers protection (OR = 0.226, 95% CI = 0.063 - 0.800, p = 0.021). Furthermore, haplotype analysis revealed rs2004776 and rs3789678 to be in strong linkage disequilibrium (D' = 1.0).

Next SNPs (rs1042713 and rs1042714) within cluster 3 found in the *ADRB2* gene were investigated for their association with the development of HTN. However, no significant differences were observed for genotypic and allelic frequencies between hypertensive cases and normotensive controls. Significant association between genetic variant (rs1042713) and HTN could also not be determined for various genetic models: genotypic AG (OR = 0.84, 95% CI = 0.50 - 1.38, p = 0.48), GG (OR = 0.66, 95% CI = 0.37 - 1.18, p = 0.16), dominant (OR = 0.76, 95% CI = 0.48 - 1.21, p = 0.26), recessive (OR = 0.73, 95% CI 0.44 - 1.21, p = 0.23), co-dominant models (OR = 0.99, 95% CI = 0.63 - 1.54, p = 0.95) and Allelic (OR = 0.76, 95% CI 0.58 - 1.01, p = 0.06). Furthermore, significant interaction was found between rs1042713 genotypes, BMI, and age on HTN in our study population.

Lastly, the low frequency of the *MTHFR* (rs1801133) T allele (5%) was also observed when compared with the C allele (95%) in both cases and controls. No significant associations were observed between rs1801133 and the risk of developing HTN in all genetic models: genotypic (OR 0.75, 95% CI 0.29 - 1.95, p = 0.56), dominant (OR 0.86, 95% CI 0.35 - 2.16, p = 0.75), co-dominant (OR 1.33, 95% CI 0.51 - 3.48, p = 0.55) and allelic (OR 0.80, 95% CI 0.49 - 1.62, p = 0.70) in logistic regression analysis. However, a significant interaction was reported among rs1801133, age, and gender (p <0.0001) with the risk of HTN.

Conclusion

Prevalence of HTN was high among the Xhosa African population of the Eastern Cape (~71%), whilst the genotype distributions of *AGT* rs3789678 and rs2004776 polymorphisms influenced the risk of developing HTN in the Xhosa South African population. Gender, age, westernized diet, education level, income status, diabetes as well as overweight/obese status were the most significant predictors of HTN. It is deciphered that for rs3789678 polymorphism, TT genotype protects against the development of HTN and for rs2004776 polymorphism TT genotype confers a 3-fold risk of developing HTN. The present study also showed that two identified *AGT* polymorphisms were in strong linkage disequilibrium. The presence of the *ACE* rs4344 polymorphism did not confer any risk of developing HTN, while *ACE* I/D

polymorphism (rs1799752) could not be detected in the studied population, and further analysis is needed on a broader group of the population.

Furthermore, this study revealed that there is no significant association between the two genetic variants (rs1042714 and rs1042713) of the *ADRB2* and *MTHFR* (rs1801133) with the risk of developing HTN. However, a potential synergistic effect occurred among rs1042713 genotypes, BMI, and age and among gender, age, and rs1801133 with HTN in our study population. However, future studies with a large sample size are required to further validate these findings and study the role of these polymorphisms (rs3789678, rs2004776, rs1801133, rs1042714, and rs1042713) in the risk of developing HTN. Additionally, more research on various other populations of South Africa and the present population is warranted to validate the current findings. Other polymorphisms in the coding and promoter regions of the candidate genes analysed should also be evaluated in various ethnic groups to determine their functional importance and impact on HTN. The discovery of these variants can aid in the understanding of genetic susceptibility factors in the population, allowing for better prevention and therapies.

Keywords

African population

Beta 2-adrenergic receptor gene

Genes

Genetic markers

Genotyping

Haplotypes

Hypertension

Methylenetetrahydrofolate reductase gene

Pharmacogenomics

Polymorphisms

Single nucleotide polymorphisms

Variation

Declarations

I declare that '*Inter-individual genetic variation and the development of hypertension in a Xhosa African population of Eastern Cape, South Africa'* is my own work, that it has not been submitted before for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged as complete references.

SIHLE EPHRAIM MABHIDA JUNE 2022

Signature……

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Dedications

I dedicate this work to my mom and dad, thank you for the unconditional love and support. I hope I have made you proud.

Published Journal Articles

Publications arising directly from this thesis

Mabhida, S.E., Muhamed, B., Sharma, J.R., Apalata, T., Nomatshila, S., Mabasa, L., Benjeddou, M., Masilela, C., Ziqubu, K., Shabalala, S. and Johnson, R., **2022**. Methylenetetrahydrofolate Reductase Polymorphism (rs1801133) and the Risk of Hypertension among African Populations: A Narrative Synthesis of Literature. Genes, 13(4), p.631.

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Publications arising indirectly from this thesis

Shabalala, S.C., Johnson, R., Basson, A.K., Ziqubu, K., Hlengwa, N., Mthembu, S.X., **Mabhida, S.E**., Mazibuko-Mbeje, S.E., Hanser, S., Cirilli, I. and Tiano, L., **2022**. Detrimental Effects of Lipid Peroxidation in Type 2 Diabetes: Exploring the Neutralizing Influence of Antioxidants. Antioxidants, 11(10), p.2071.

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List of abbreviations

AHA - American Heart Association

ACE - Angiotensin-converting enzyme

AGT - Angiotensinogen

BH adjusted p-value - Benjamin-Hochberg adjusted probability value

ADRB2 - Beta 2-adrenergic receptor gene

BP - Blood pressure

BMI - Body mass index

CVD - Cardiovascular Disease

CI - Confidence Intervals

DBP - Diastolic blood pressure

FDA - Food and Drug Administration

GO - Gene ontology

GWAS - Genome-wide Association Study

HWE - Hardy-Weinberg Equilibrium

HuGENet - Human Genome Epidemiology Network

HTN - Hypertension

ICD - International Classification of Disease

KEGG - Kyoto Encyclopedia of Genes and Genomes

LD - Linkage disequilibrium

LMICs - Low and middle-income countries

MALDI-TOF - Matrix-assisted laser desorption/ionisation time of flight

MeSH - Medical Subject Headings

MTHFR - Methylenetetrahydrofolate reductase gene

NCI - National Cancer Institute

NOS3 - Nitric oxide synthase

NCDs - Non-communicable diseases

OR - Odds ratios

OMIM - Online Mendelian Inheritance in Man

ATP2B1 - Plasma membrane calcium-transporting ATPase 1

PPIs - Predict protein-protein interactions

PRISMA - Preferred Reporting Items for Systematic Review

q-RT-PCR - Quantitative real-time polymerase chain reaction

RAAS - Renin-angiotensin-aldosterone system

REDCap - Research Electronic Data Capture System

RH - Resistant hypertension

SNPs - Single nucleotide polymorphisms

SES - Socioeconomic status

SANHANES - South African National Health and Nutrition Examination Survey

SPSS - Statistical package for social sciences

SNOMED CT - Systematized Nomenclature of Medicine - Clinical Terms

SNS - Sympathetic Nervous System

SBP - Systolic blood pressure

TRH - Treatment-resistant hypertension

T2DM - Type 2 Diabetes Mellitus

WC - Waist Circumference

WHR - Waist Hip Ratio

WHO - World Health Organisation

Preface

The research ethic for this study was approved by the Senate Research Committee of the University of the Western Cape (Ethics clearance number BM19/8/19), Water Sisulu University (073/15), and the South African Medical Research Council (EC028-8/2020). The study was conducted according to the principles expressed in the Helsinki Declaration. Free and written informed consent in both English and IsiXhosa was obtained from all participants of the present study, as previously reported. This thesis is comprised of eight chapters arranged in the following order: **Chapter one** (introduction), **Chapter two** (Literature review), **Chapter three** (systematic review), **Chapter four** (narrative synthesis of literature review) **Chapter five to eight**, (SNPs association studies conducted in article-ready format) and **Chapter nine** (conclusion and future prospects).

The thesis is structured and formatted according to the PhD by publication guidelines of the University of the Western Cape Therefore, this doctoral thesis incorporates a collection of published journal articles and manuscripts under review, together with an introduction and summary of each article as part of a PhD by publication. Manuscripts are presented based on the guidelines of the respective journals. Manuscripts presented in this thesis were submitted to open access journals. Therefore, the manuscripts are accessible online without any restrictions. Correspondence between the authors and reviewers are published alongside the articles in their respective journals.

Submitting a PhD thesis in this format allows the student to develop a research identity earlier on in their academic carriers. It also contributes to the early dissemination of new knowledge

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CHAPTER ONE:

1.1 Introduction

According to the World Health Organisation (WHO), hypertension (HTN) affects about 1.4 billion individuals globally with 74 million cases on the African continent [1, 2]. Indeed, it is a leading cause of death, and the prevalence has been dramatically increasing over the past two decades especially in developing countries [1, 3]. As a result, HTN remains the most common life-threatening risk factor contributing to the high mortality rate in Africa with almost a million total deaths per year [4, 5]. In fact, the estimated prevalence of HTN varies from 5 to 58% in Africa, with South Africa, having the highest prevalence range between 27 to 58%. [6-8]. These numbers are expected to rise dramatically soon if no effective intervention strategies are implemented timeously. Many factors such as the poor efficacy of the treatments, the failure to adhere to the treatment [9, 10], various environmental and demographic factors (age, race, gender, poor diet, alcohol consumption, cigarette smoking, and poor health system) majorly contribute to the rising prevalence of HTN in South Africa, particularly in rural areas [11, 12].

Several risk factors contributing to the development of HTN are categorized as modifiable and non-modifiable risk factors. The identification of modifiable (alcohol consumption, tobacco use, poor diet, and lack of exercise) and non-modified risk factors (genetics, sex, and ethnicity) of HTN is essential in order to identify high-risk groups that require special attention and proper treatment [3, 13]. Among the plethora of non-modifiable risk factors, genetic factors play a profound role in the etiology of HTN. [14, 15]. A growing body of evidence supports utilizing informative genetic variants to identify HTN subtypes [16-18], which emphasises the importance of deciphering the biological basis of any disease and the role played by genetic variations in the development and progression of a disease. Over the past several years, numerous researchers have uncovered the significant role of inter-individual genetic variations such as single nucleotide polymorphisms (SNPs) in the development and progression of HTN [19-21]. Knowledge of an individual's genetic predisposition to HTN could help to implement preventive measures as well as formulate effective therapeutics at an early stage [22]. Additionally, pharmacogenomic information is further utilised in the development of personalised medication regimens, which will surely improve therapeutic responses and assist in reducing healthcare costs [23]. However, exploring the role of these genetic variations in developing the risk of HTN has been challenging due to the inconsistent findings in different studies. Due to these variations, further studies are needed to confirm the association between various genetic polymorphisms and the risk of HTN. To the best of our knowledge, there are very few studies that have investigated genomic variants associated with HTN in Africa. Further genetic analysis of diverse ethnic groups is required to examine whether these risk variants can be used as biomarkers to predict the development of HTN in an African population.

African residents are characterized by high genetic diversity and low levels of linkage disequilibrium among loci when compared to populations from other countries. For this reason, there are ongoing studies to understand genomic diversity within African populations. Available studies suggest that from a genetic standpoint, there is no SNP database that can be used to treat or predict the development of HTN in an African population [24]. Moreover, South Africa is one of the Sub-Saharan African countries with a diverse population. Available studies suggest that South African populations have unique genetic profiles which include novel and rare variants, with allele frequencies differing from other African populations in relevant genes [25, 26]. Therefore, the aim of the study is to investigate inter-individual genetic variants and their association with the risk of developing HTN in the indigenous South African

1.2 Aim and Objectives of the study

Aim: The aim of the study is to generate SNPs that are relevant within an African population and to investigate their inter-individual genetic variation and the development of HTN in a Xhosa South African population.

Objective 1: To assess the prevalence and associated risk factors of hypertension in Xhosa South African population.

Objective 2: To perform in silico identification of genetic variants potentially associated with hypertension treatment response

Objective 3: To characterise potential drug gene targets linked to identified genetic variants in terms of the drug-gene interactions, co-expression and pathway analysis to assess their involvement in treatment response.

Objective 4: To investigate the association of methylenetetrahydrofolate Reductase Gene Polymorphism (rs1801133) and Risk of Hypertension Disease in African Population: A Narrative Review.

Objective 5: To investigate the role of identified genetic variants in predicting hypertension in Xhosa South African population.

1.3 Structure of the Dissertation

The thesis has been organized in the following chapters

Chapter 1: Introduction - the chapter introduces the topic and gives a brief background of the study

Chapter 2: Literature review - reviews literature relevant to the study

Chapter 3: Systematic review (quantitative) - reviews literature relevant to the study and systematically extracts and discusses African evidence on the effect of genetic variations in hypertensive patients and performs an *in-silico* evaluation. The chapter is now published in the high impact factor journal (Genes, IF 4.1) and structured in accordance with its specific format. This chapter was designed to address **objectives 2 and 3**.

Chapter 4: Narrative review (qualitative) - the chapter serves as a follow-up study from chapter 3 and the purpose of this chapter sought to gather and analyse the available genetic evidence on the association between *MTHFR* (rs1801133) and the risk of developing HTN in an African population and further compare the evidence with global data. The chapter is now published in the high impact factor journal (Genes, IF 4.1) and structured in accordance with its specific format. This chapter was designed to address **objective 4**.

Chapter 5: Assess the prevalence and associated risk factors of HTN in the indigenous Xhosa South African population. The chapter in now published in the high impact factor journal (International Journal of Environmental Research and Public Health, IF 3.390) This chapter was designed to address **objective 1**.

Chapters 6-8: Report on the identified genetic variants in predicting HTN in the Xhosa South African population. Each of the chapters is formatted in the form of a journal article and includes the abstract, introduction, materials and methods, results, discussion, and conclusion. These chapters are structured in accordance with the specific journal format. These chapters were designed to address **objective 5**.

Chapter 9: General conclusion and future work

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CHAPTER TWO

Literature Review

2.1 An overview of hypertension and its history

Hypertension (HTN) is well-known as an asymptomatic disorder characterized by persistent elevated systolic/diastolic blood pressure (SBP and DBP) of ≥130/80 mmHg respectively. It was firstly discovered in 1896 by Scipione Riva-Rocci, an Italian physician who measured the peak of SBP by cuff pressure of the mercury sphygmomanometer [1]. In 1905, Nikolai Korotkoff a physician from Russia performed an experimental study to demonstrate the artery sounds that are used to define SBP and DBP measurements and clinical recording of blood pressure (BP) [2]. Henceforth, since then, this has become the standard method to diagnose HTN in patients in the past century. It has been reported that the main risk factors contributing to the development of HTN include modifiable (alcohol consumption, tobacco use, poor diet, and lack of exercise) and non-modifiable (genetics, sex, and ethnicity) risk factors in different populations. Thus, identifying modifiable and non-modified risk factors of HTN is essential in order to identify high-risk groups that require special attention and proper treatment [3, 4]. There is evidence that family history is an important non-modifiable risk factor for HTN, and other cardiovascular diseases (CVD) and numerous reports have shown that BP is more similar between relatives than between unrelated individuals. Several types of HTN such as essential, secondary, malignant, isolated systolic, and uncontrolled HTN have been identified as shown and defined in table 2.1 [5, 6]. Essential HTN is defined as a complex disorder that can be attributed to the interaction between genes/genetic variants and environmental factors, which accounts for 90–95% of all susceptible cases [7, 8]. And the remaining 5-10% of patients, HTN is referred to as secondary HTN, which is also known as arterial HTN. Secondary HTN is a less common form of HTN that occurs because of a specific underlying condition or disorders such as sleep apnea, tumors, and kidney failure [9].

Table 2.1. Illustrates the common types of HTN with a brief description for each type.

Type of hypertension	Prevalence (%)	Description	BP range (mmHg)	Reference
Essential (primary) hypertension	90–95%	Chronic elevation in BP with no underlying disease	≥130-139/80-89	[10]
Secondary hypertension	5-10%	Chronic elevation in BP due to underlying pathology	≥130-139/80-89	[11]
Malignant hypertension	n/a	When BP is severely elevated and causes an organ damage	≥130-139/80-89	[12]

Isolated systolic hypertension	33.8%	Common in the elderly due to the loss of elasticity of major blood arteries	≥130-139/80-89	[13]
Treatment-resistant hypertension	20-30%	Blood pressure that remains above goal despite taking >3 anti-HTN drugs from diff classes taken at max dose, one of which should be a diuretic. TRH might have a genetic component	≥140/90	[14]

2.2 Classification of blood pressure

There are several guidelines used to define the onset and severe cases of HTN. For instance, the South African Guidelines define HTN for adults (≥ 18 years,) as an SBP ≥ 140 or DBP ≥ 90 mm Hg on three separate readings taken 5mins apart [15, 16]. The optimal BP is a value of <120-129 and <80 mmHg (SBP and DBP). High normal is BP levels from 130–139 or 80–89 mmHg (SBP or DBP) as shown in table 2.2. According to South African Guidelines, the optimal and elevated BP subjects is at higher CVD risk and are also at risk of developing HTN, but do not require drug treatment. HTN is highly prevalent in South Africa, a large proportion of public healthcare budgets are spent screening, treating, and controlling HTN due to a late diagnosis of the early onset and severe cases of HTN [17]. Thus, there is sufficient evidence to support the critical need for the review or amendments of the current South African guidelines to be SBP ≥ 130 or DBP ≥ 80 mm Hg. The use of a broad HTN definition such as American Heart Association guidelines (AHA) increases not only the number of eligible cases but also helps in early treatment and the diagnosis of the early onset of the disease but has been assessed regarding their applicability in developing countries [18]. Therefore, establishing cost-effective best practice guidelines for HTN diagnosis and treatment requires further guideline amendments. Such amendments will be essential if South Africa is to make progress in its efforts to implement universal healthcare. The AHA classify HTN as stage 1 (SBP: 130–139 or DBP: 80-89 mmHg) or stage 2 (SBP: ≥ 140 or DBP: 80-89 mmHg). The differences in BP thresholds between the South African and AHA guidelines are outlined in table 2.2.

Table 2.2. Classification of hypertension

	South African Guidelines			American Heart Association Guidelines		
Blood Pressure Category	***Systolic Blood Pressure***		***diastolic Blood Pressure***	***Systolic Blood Pressure***		***diastolic Blood Pressure***
Normal	<120	and	<80	<120	and	<80
Optional	120-129	and	<80	n/a	n/a	n/a
Elevated	130-139	or	80-89	120-129	and	<80
Hypertension						
Grade/Stage 1	140-159	or	90-99	130-139	or	80-89
Grade/Stage 2	160-179	or	100-109	≥140	or	≥90
Grade 3	≥180	or	≥110	n/a	n/a	n/a

2.3 Prevalence of hypertension

The prevalence of HTN has been increasing alarmingly in developed and undeveloped countries over the last two decades [3, 19]. Literature evidence has reported that over 1 billion people are affected by HTN and that accounts for 9.4 million annual deaths globally [20]. In Africa, where health resources are scarce, the estimated prevalence of HTN varies from 5 to 53% affecting approximately 100 million individuals with an estimate of one million deaths per year [21, 22]. Price et al, [23] and Mawaw et al, [24] reported that HTN is one of the most common causes of morbidity and mortality on the African continent. According to the World Health Organisation (WHO), the prevalence of HTN in South Africa varies between 27 -58% [21, 25, 26]. However, to date, very few studies have assessed the prevalence of HTN in the rural parts of South Africa such as Mthatha, Eastern Cape, where a prevalence as high as 75% was reported by Abeniyi et al [26] and confirmed by Sharma et al, [3, 26].

2.4 Pathophysiological mechanisms of hypertension

HTN is a complex disorder with its precise pathophysiology remaining a subject of debate. The kidney is the most contributing organ to the hypertensive processes, [27] and HTN involves the interaction of multiple organ systems and numerous mechanisms of independent or interdependent pathways. Factors that play an important role in the pathogenesis of HTN include activation of neurohormonal systems such as the sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS), obesity, and genetics [28].

2.4.1 Sympathetic nervous system

The sympathetic nervous system is a key regulator and major contributor to the pathogenesis and pathophysiology of HTN [8]. Sympathetic over-activity occurs in early primary HTN and in several other forms of established forms of HTN, including HTN associated with obesity, sleep apnea, early type 2 diabetes mellitus and prediabetes, chronic kidney disease, and heart failure. Both central and peripheral mechanisms have involved an increase in sympathetic activity. Emotional and physical stress activates sympathoadrenal activity and increases BP.

2.4.2 Renin-angiotensin-aldosterone system

The RAAS plays a vital role in the maintenance of normal BP and is activated by dual mechanisms and stimulation of the SNS [7, 29]. RAAS controls BP, water retention, sodium homeostasis, oxidative stress, and inflammation through effects that are coordinated via combined mechanisms in the kidney, and central nervous system [30] involving the activation of various key proteins [31]. Proteins such as angiotensinogen (*AGT*), angiotensin-converting enzyme (*ACE*), renin, angiotensin I, angiotensin II, angiotensin receptor type I, and angiotensin receptor type II are significant components of RAAS. Briefly, the effects of RAAS

are mediated by the renin-related conversion of angiotensinogen into angiotensin I, which is further cleaved by the *ACE* to produce angiotensin II (Ang II). Ang II, which is the final effector of the system, stimulates the angiotensin II type 1 receptor (AT1R) present in the vasculature, kidney, and central nervous system, resulting in vasoconstriction and inhibiting bradykinin-modulated vasodilation, resulting in elevated BP (Figure 2.1) [30].

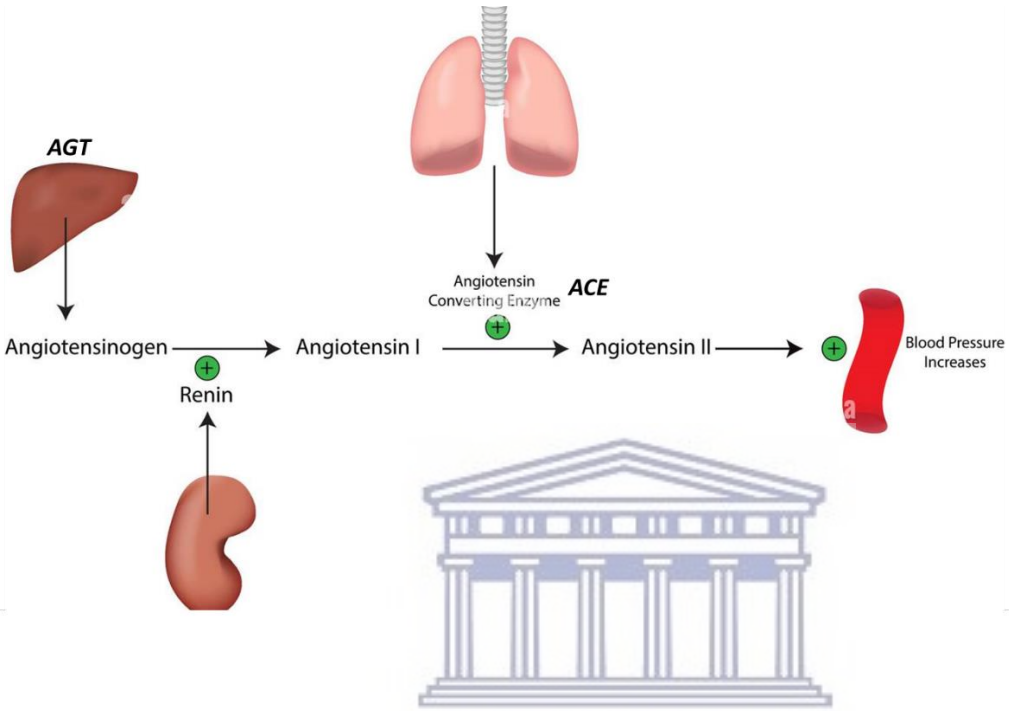

Figure 2.1: The renin-angiotensin system pathophysiological mechanism pathway in hypertension [30]

Furthermore, it has been reported that an increase in Ang II may cause increased oxidative stress and block catecholamine bindings to the adrenergic receptors. This, cause an increase of catecholamines hormone in the blood system which is known to induce vascular resistance and subsequently stimulate an increased BP (Figure 2.2). Therefore, the maintenance of normal BP is dependent on the balance between cardiac output and systemic vascular resistance. Medications for HTN targeting proteins or genes such as *AGT, ACE,* renin, (RAAS), and centrally active adrenergic receptors (*ADRB2* gene) of the SNS effectively lower BP in most patients [32].

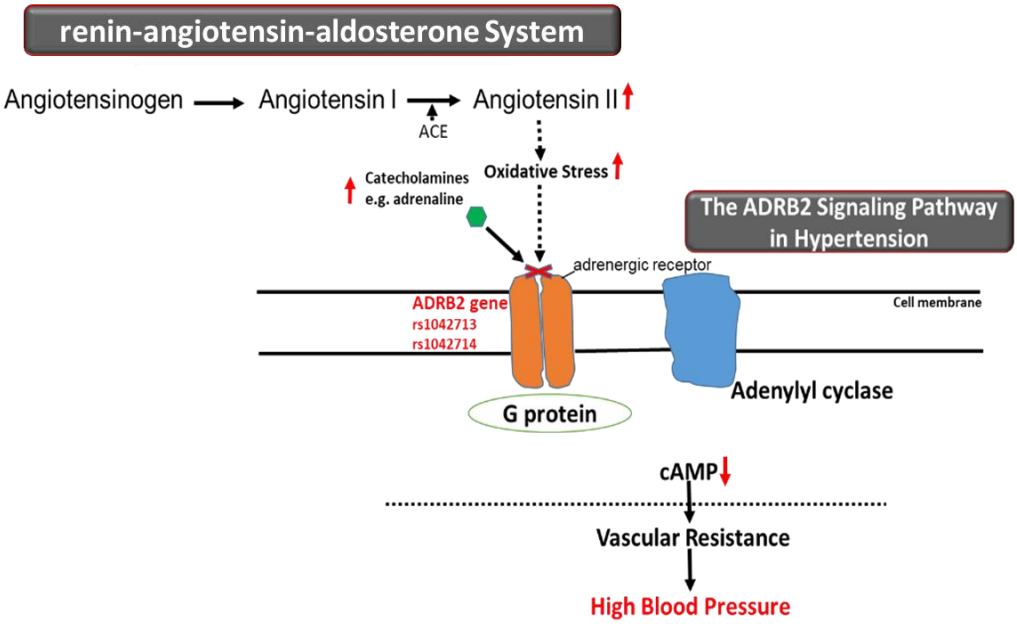

Figure 2.2: The sympathetic nervous system and renin-angiotensin system pathophysiological mechanism pathway in hypertension [32].

2.4.3 Hormonal mechanisms

Evidence from the literature has shown that sex hormones can influence BP regulation through activation of the RAAS, reducing the excretion of sodium in the urine, and through increased endothelin, and oxidative stress [33, 34]. Men are thought to be at high risk for HTN at earlier ages than women, and these mechanisms are not likely to be as simple as the presence of testosterone since androgen levels drop in men with HTN [35]. In fact, many investigators now believe that it is the reduction in androgen levels that may exacerbate HTN in men [35]. Premenopausal hypertensive women with regular menstrual cycles appear to have lower plasma estradiol levels than normotensive women and the protective function of oestrogen has been suggested to account for approximately fifteen years of delay in presentation of HTN in women compared with men [35]. Oestrogen activates the counterparts of the RAAS as well as improves endothelial function. Moreover, lower plasma renin levels have also been reported in women with primary HTN compared with normotensive women and men. Plasma renin activity increases after menopause and the upregulation of angiotensin II receptors and downregulation of angiotensin I receptors after menopause might influence response to therapy. Also, endothelin levels and oxidative stress increase after menopause and may affect BP through increased sodium reabsorption and vasoconstriction [30, 36].

2.5 Risk factors of hypertension

2.5.1. Gender

It has been reported that gender may influence the control and prevalence of HTN in different age groups [37]. Literature evidence suggests that women are more likely to develop HTN at the age of 75 years and above [38, 39]. While men it has been reported to have a much higher prevalence of HTN between the age of 20 and 65 years. Data evidence suggests that multiple sex-specific processes such as estrogen receptors and SNS activation, pregnancy complications, and a combination of modifiable factors predispose women to the development of HTN. These factors are linked with a decline in endothelial function occurring later in women's lives, which is in part related to endogenous estrogen stimulation of nitric oxide synthesis until menopause [40]. Most hypertensive women develop adverse pathophysiologic consequences such as left ventricular hypertrophy, diastolic dysfunction, heart failure, increased arterial stiffness, diabetes, and chronic kidney disease in comparison to men [41]. Previous studies on multivariable analyses in the older African population demonstrated that females are associated with a higher prevalence of HTN, but the difference was not statistically significant (Male 41% vs. Female 42%) [3, 37, 42].

2.5.2 Age

BP increases with age, and this is mostly associated with physiological changes in the arteries and especially with large artery stiffness. In individuals aged 50 and above, the most essential predictor of risk is increased pulse pressure due to decreased diastolic DBP and increased SBP [43]. Based on the Framingham study, it is estimated that about 90% of normotensive persons aged 55 years and over will develop HTN in their lifetime [44]. In Africa, about 57% of adults aged 50 years or above have HTN. Similarly, it is estimated that 55.2% of adults aged 55 years and older are hypertensive, as reviewed elsewhere [43]. In young men, the independent predictors of HTN were adiposity, high uric acid level, high resting heart rate, and hypertriglyceridemia. Moreover, In young women, the same predictors were observed including alcohol consumption [45]. In the same cohort, they found a prevalence of 14.2% and 12.9% in men and women of the same age respectively [45]. Furthermore, Coronary Artery Risk Development in Young Adults Study (CARDIA) has reported the increasing cases of HTN among the young adult population at an age of 18-30 years [46]. This association showed the increasing level of HTN prevalence in early adulthood, suggesting increasing future cardiovascular events.

2.5.3 Race

Race reflects differences in social and cultural influences such as health behaviors, access to health care, and environmental exposures that may affect BP [80]. A recent study showed that HTN is more prevalent

among African Americans (60%) in comparison to Caucasians (38%), Hispanics (42%), and Chinese patients (39%). It was further demonstrated that Caucasian participants had a lower rate of uncontrolled HTN in comparison to all the study groups [81]. Furthermore, individuals of African ancestry were recently shown to present with the earliest onset of HTN compared to Caucasian, regardless of equal awareness and treatment efforts [47]. A study by Geronimus and colleagues found that Africans at an early age have nearly twice the HTN prevalence rates compared to Caucasian counterparts [47, 48]. In black Africans, HTN has been reported to be associated with a higher rate of stroke, end-stage renal disease, and heart failure compared to Caucasian people. A study by Kramer et, al [49] demonstrated that Caucasian patients in comparison with African Americans, Chinese patients, and Hispanics had a lower rate of uncontrolled HTN. However, in their study, HTN was more prevalent among African Americans when compared to all other study groups. The data from the CARDIA study showed that depressive states were predictive of a higher incidence of HTN in young adults, particularly in young black African patients [19]. Recently, the Jackson hearts study was conducted to assess racial differences in young adults and their role in the development of HTN. It was found that indeed, race has a significant factor associated with the excess HTN burden in Africans [50, 51].

2.5.4 Poor diet

The close relationship between HTN and dietary sodium intake is widely recognized and supported by many studies [52, 53]. Well-balanced diet not only decreases BP and the incidence of HTN but is also associated with a reduction in morbidity and mortality from CVD. A prolonged modest reduction in salt intake induces a relevant fall in BP in both hypertensive and normotensive individuals, irrespective of sex and ethnic group, with a significant fall in SBP attributed to low dietary salt intake [53]. The high sodium intake and the increase in BP levels are related to water retention, an increase in systemic peripheral resistance, and alterations in the endothelial function, structure, and function of large elastic arteries, sympathetic activity, and the autonomic neuronal modulation of the cardiovascular system [54]. An unhealthy diet is a major modification behavioral risk factor in the development of overweight and HTN [55]. Although a variety of dietary recommendations for the management and prevention of obesity and HTN have been proposed, evidence is inconsistent and varies between measures of dietary intake used [56]. With 65 to 75% of the incidence of HTN directly related to overweight, the role of diet in obesity associated with HTN is an important consideration for the development of dietary guidelines [56].

2.5.5 Overweight/Obesity

Excess body weight is one of the leading risk factors contributing to the global burden of cardiovascular disease globally. Being overweight has been consistently associated with HTN risk [55]. Based on

population studies, the risk estimate indicates that at least two-thirds of the prevalence of HTN can be directly attributed to being overweight. It has been demonstrated that obesity is associated with overactivation of both the sympathetic nervous system and the renin-angiotensin system, changes in the adipose-derived cytokines, insulin resistance, the structural and functional renal alterations contributing to the emergence of HTN.-Various studies [57-59] have reported a positive association between obesity and HTN in different countries of sub-Saharan Africa, and it has been reported recently that being overweight doubles the odds of developing HTN [3]. In contrast weight loss is known to be associated with the reduction of BP [60]. Even a modest reduction in body weight can cause a meaningful reduction in the activity of the RAAS, the result of which is a reduction in BP[61]. Furthermore, weight loss has been shown to improve endothelial function, decrease SNS activity and improve baroreflex function [62]. There is growing evidence that the prevalence of HTN in part due to obesity may contribute to the progression of chronic kidney diseases [63].

2.5.6 Alcohol consumption

Various epidemiological, preclinical, and clinical studies have demonstrated the association between alcohol consumption and HTN. However, current research suggests that low to moderate drinking is beneficial to the cardiovascular system and demonstrates a modest decrease in BP [64] epidemiological studies have reported that more than three drinks per day are associated with a high risk of HTN and CVD [65, 66]. In South Africa, heavy drinking is prevalent among more than 40% of young male individuals, leading to the loss of more than 10,000 lives annually [67, 68]. Although the mechanism by which alcohol increases the level of BP is not well understood, it has been suggested that stimulation of the RAAS, imbalance of the central nervous system, and inhibition of endothelium-dependent nitric oxide production due to inflammation and oxidative stress can be the possible mechanisms. Above all, the inhibition of endothelium-dependent nitric oxide production is the major contributor to alcohol-induced HTN [69, 70]. In addition to the reduction of alcohol intake, physical exercise is one of the most important strategies to prevent chronic alcohol-induced HTN [70].

2.5.7 Cigarette smoking

Smoking is one of the major modifiable risk factors contributing to the global burden of HTN [71]. In Africa, where HTN is highly prevalent, the number of active smoking in hypertensive patients is increasing daily, with the heaviest burden in Northern Africa. As per WHO, the prevalence of smoking in the African population is around 16%, however, there is a wide variation of prevalence estimates from 1.8%-25.8% among different African countries [72, 73]. Smoking prevention and cessation are one of the most crucial components in the management of HTN, which can help to prevent a large number of HTN deaths [71].

However, the association between smoking and BP is not univocal, with some studies reporting a positive while others showed an inverse association [74-77]. Impairment of endothelial function and arterial stiffness are smoking-related major determinants of the atherothrombotic process leading to serious cardiovascular events [78]. In addition, it has also been concluded that smoking causes major changes in forearm haemodynamics involving both small and large arteries, damage to the endothelium, and has been implicated to be important in the pathophysiology of HTN [79]. Furthermore, smoking affects arterial stiffness and wave reflection which may lead to a greater detrimental effect on central BP, which is closely related to target organ damage than brachial BP [79, 80]. Thus, hypertensive smokers are more likely to develop severe forms of HTN such as malignant and renovascular HTN, which occur as a result of accelerated atherosclerosis [12].

2.5.8 Epigenetics

In recent years, altered epigenetic mechanisms have been postulated to underlie the development of many diseases, including HTN [81, 82]. Epigenetics is the study of heritable changes in gene expression or phenotype that occurs without changes in the underlying DNA sequence [83]. Each cell has a unique epigenetic signature that controls normal growth and development, eventually resulting in the phenotype of the cell and tissue. Genetics and environmental factors such as unhealthy diets and lack of physical activity induce epigenetic modifications that affect these biological systems [84], making them important pathogenic mechanisms in complex multifactorial diseases. Due to their reversible nature, epigenetic changes may provide a window of opportunity for intervention strategies to prevent or reverse HTN and improve health outcomes. In addition, accumulating evidence suggests that intrauterine environmental exposure leads to persistent epigenetic modifications in developmentally important genes, that predispose to adverse health outcomes [85, 86]. Several studies focusing on the developmental origin of health and disease and metabolic programming have identified a link between environmental influences and epigenetic mechanisms such as DNA methylation, histone modifications, and non-coding RNA regulation (miRNA), suggesting an important role of these epigenetic mechanisms as metabolic and developmental regulators during HTN complications [81, 85, 87].

2.5.9 Genetics

There has been a long-standing interest in understanding non-modifiable factors such as sex, ethnicity, and genetics that are known to contribute to the development and the progression of HTN. Genetic variation plays a huge role in the pathophysiology of HTN contributing to 30-50% of BP variation. However, it has been challenging to understand the role of genetic factors and known genetic variations are only able to explain only 3% of BP variation, emphasizing the fact that many genetic variants are still needed to be identified. Various Twin- and family-based studies have indicated that as much as 30–50% of the variance

in BP readings may be heritable, while various rare disorders have led to the identification of specific mendelian mutations. Over the time several studies including, candidate gene, genome-wide association, and twin/family studies have been performed to investigate the association of various genes and their related Single nucleotide polymorphisms (SNPs) with the increasing risk of developing HTN [88].

2.5.9.1 Twin/family studies in hypertension

Twin studies present an important opportunity to estimate the influence of genetics and the environment at the aggregate level. Correlations among relatives can arise from a combination of shared genes and shared environments. The classical twin study is based on the monozygotic twin's information which is known to be genetically identical and share all their genes, as opposed to dizygotic twins who share 50% of the same genes (the same as siblings) [89]. Twin estimates of heritability are consistently high, suggesting genetic factors contribute approximately 70-90% to refractive error variance [90, 91]. However, twin studies will provide an estimate of heritability that is population-specific, and therefore, a high heritability estimate may be seen in one population that is not generalizable to another [92].

Twin studies have more power to detect heritable effects than family studies [93]. Traditional twin/family studies and genome-wide association studies (GWAS) are complementary methods for behavioral genetic research. Estimates of genetic co-variance from these two approaches have different assumptions, require different sample sizes for adequate power, and have distinct interpretations. Data from both approaches can be used to test causal models. Family data can be used as control for confounds in GWAS and probe gene-environment interaction. Twin studies examining BP have since been performed in numerous populations and ethnic groups, demonstrating the universal applicability of twin data in humans [94]. However, there is a probability of overestimated heritability in twin studies due to violations of shared environment assumptions, poor phenotyping practices in the control group, unaccountability of epistasis, gene-gene and gene-environment interactions, and other contributors of phenotype changes that may account for underestimated heritability in GWAS [95, 96].

2.5.9.2 Genome-wide association studies in hypertension

It is almost 15-years since the first genome-wide association study for HTN, and after a slow start, there is now over 1000 BP loci explaining ~6% of the SNP-based heritability. Genome-wide association study is currently the most commonly used method for identifying genetic variants associated with complex diseases [97]. The technique relies on comparing SNPs across the genome of cases and controls to determine the variants that are associated with the diseases such as HTN [97]. Furthermore, GWAS studies have shown that genetic factors are related not only to BP elevation but also to inter-individual variability in response to antihypertensive treatment [98-100]. GWAS has identified more than 100 SNPs that are associated with HTN in different ethnicities [101]. Due to variations across different ethnic groups, further studies are

needed to confirm the association between risk alleles and HTN. Further analysis of diverse ethnic groups is required to examine whether these risk variants can be used as biomarkers to predict the development of HTN. GWAS has been considered better as compared to linkage analysis and candidate gene studies because of the unbiased results, involvement of large sample size, and ability to metanalysis for the improvement of statistical power [102]. In the first GWAS for HTN, no significant association was reported with any identified loci [103, 104]. However, in 2009, GWAS identified the association between the plasma membrane calcium-transporting ATPase 1 (*ATP2B1*) polymorphisms (rs2681472, rs17249754, rs2681492) and HTN in various populations [100, 104]. This gene encodes for plasma membrane calcium-dependent ATPase, which handles calcium pumping to the extracellular compartment [105]. This result was replicated in a Korean cohort of >8000 individuals [100, 104] and in a large European ancestry cohort of nearly 30,000 individuals [101]. Over the following years, many investigators using GWAS have identified loci for the quantitative traits of systolic, diastolic, and pulse pressure [106, 107], facilitating the discovery of novel BP pathways. While GWAS studies investigate genetic variants spanning the entire genome, candidate gene studies limit the analysis to a relatively few numbers of genes and their SNP related [107].

2.5.9.3 Candidate gene studies in hypertension

Candidate gene approaches evaluate specific genetic variants hypothesized to be related to a particular disease such as HTN. This resulted in a surge of candidate gene studies reporting on the genetic association between HTN and respective gene variants [108]. In the candidate gene approach, there are several selection strategies used to identify candidate genes and their variants. These include candidate regions identified through results from association studies and meta-analysis, linkage studies, whole-genome approaches, candidate genes validated through functional analysis, sequence homologies with functional genes in animal models, results from gene expression studies, and knowledge of the pathophysiological mechanisms of the HTN. Candidate gene sample sizes are typically in the hundreds, orders of magnitude smaller than typical GWAS sample sizes, yet most published candidate gene studies reported significant associations [109, 110]. There are numerous important steps to follow in a candidate gene study including the scientific protocol, study design, HTN definition, candidate gene of interest and variant selection, haplotype analysis and linkage disequilibrium, sample size estimation, statistical significance, testing of statistical association, and functional analysis (Figure 2.3).

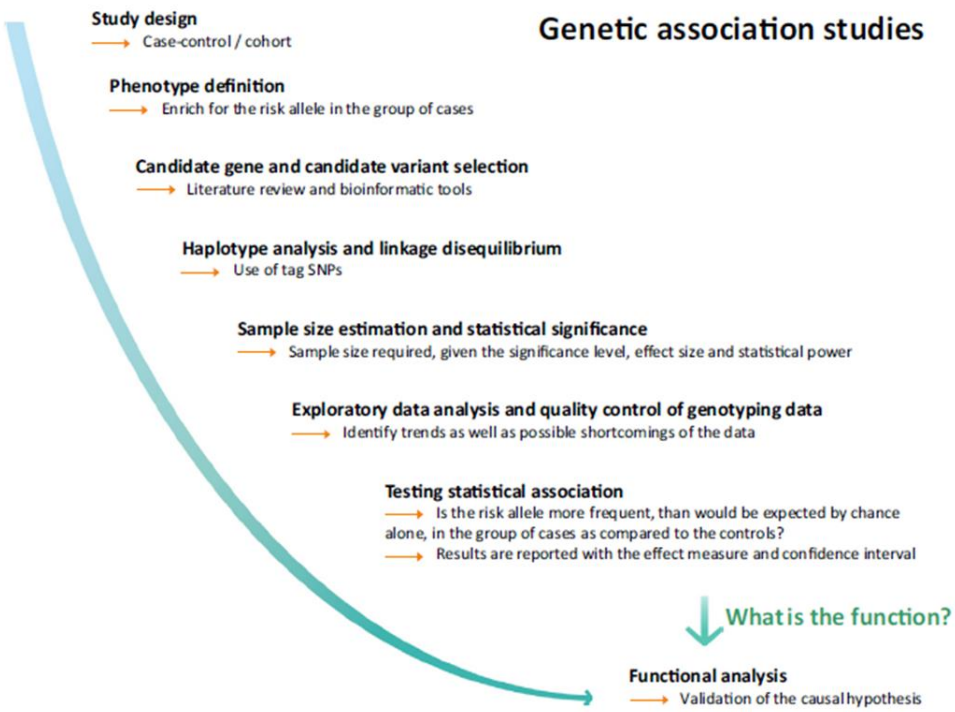

Figure 2.3. Steps to candidate gene association studies [103].

Several studies have been conducted to investigate genes that are involved in BP regulation in HTN. Various studies have utilised the candidate gene approach including genes of the RAAS, cell signaling pathways, and ANS. Genetic variations in the key genes of the RAAS such as the angiotensinogen (AGT), angiotensin-converting enzyme (ACE), angiotensin II-type 1 receptor (AGTR1), aldosterone (CYP11B2), and renin (REN) have been reported to be associated with the development of HTN [111]. However, the challenges faced by the candidate gene studies in HTN include poorly designed studies, a small sample size, and contradictory findings. Furthermore, these studies have proven difficult to replicate in other populations. This demonstrates the degree of heterogeneity in HTN etiology, interactions between environmental and genetic factors in different populations lead to quantitatively different influences of genetic variants.

2.5.9.4 Inter-individual genetic variations in an African population

Single nucleotide polymorphisms are single-base variations in the genome that occur at different frequencies in the population. SNPs that are distributed across the genome are tested for their association with diseases such as HTN. The polymorphisms in various genes within RAAS have been reported to have a significant association with the development of HTN. For example, *ACE* and *AGT* genes have been investigated in various ethnic populations, and accumulating studies report that polymorphisms in these genes may be associated with an increased risk of developing HTN [112-114]. The *ACE* and *AGT* genes

encode for *AGT* and *ACE* protein, respectively [115]. SNPs within the *AGT* gene, such as rs3789678 and rs2004776, have been investigated for their causal role in HTN [116]. Furthermore, two polymorphisms in the *ACE* gene (rs1799752) and the rs4344 have also been reported to be associated with HTN in various populations [117, 118]. To date, only one study [114] reported an association of rs2004776 SNP with HTN in an African population (Uganda). However, other studies performed on African populations exhibited no association of *AGT* gene polymorphism with the risk of developing HTN [119-122]. *ACE* polymorphisms have been widely studied in various African populations [123-125], and it has been demonstrated that rs1799752 could be a potential genetic predictor for the risk of developing HTN [126, 127] however, conflicting results have been reported in other studies [123, 128, 129].

Furthermore, significant evidence in candidate gene association studies, supporting the genetic susceptibility for HTN has been provided by various studies in different ethnic groups in African populations. [19, 130-132]. Such studies reported that SNPs identified in various genes such as *ADRB2* (rs1042713 and rs1042714), *AGT* (rs2004776) [132], *NOS3* (rs1799983, rs61722009) [131, 133] and *SCNN1B* (rs149868979) [134] genes were correlated with HTN development in different parts of Africa as shown in Table 2.3. However, the results were inconsistent and we will expand on this topic in the following chapters (three and four) [135, 136].

Table 2.3. Hypertension and single-nucleotide polymorphisms association in Africa.

GENE	SNP	ASSOCIATION ($P < 0.05$)	COUNTRY	AUTHOR YEAR
ACE	rs1799752	Yes	Egypt	Zawilla et al., 2014 [44]
AGT	rs2004776	Yes	Uganda	Kayima et al., 2017 [33]
		Yes	Egypt	Farrag et al., 2011 [42]
		Yes	Ghana	Williams et al., 2004 [43]
		Yes	Egypt	Zawilla et al., 2014 [44]
		Yes	Egypt	Badr et al., 2012 [45]
		Yes	Egypt	Bessa et al., 2009 [46]
NOS3	rs1799983	Yes	Algeria	Amrani-Midoun et al., 2019 [47]
		Yes	Morocco	Nassereddine et al., 2018 [25]
	intron 4 VNTR	Yes		
	rs1799983	Yes	Tunisia	ALrefai et al., 2016 [39]
	rs2070744	Yes	Tunisia	Jemaa et al., 2011 [49]
	rs61722009	Yes	Tunisia	Jemaa et al., 2009 [51]
		Yes	Algeria	Lardjam-Hetraf et al.,2015 [53]
		Yes	Morocco	Nassereddine et al., 2015 [54]
	rs2681472	Yes	Uganda	Kayima et al., 2017 [33]
	rs17249754	Yes	Algeria	Lardjam-Hetraf et al.,2015 [53]
	rs2681492	Yes	Uganda	Kayima et al., 2017 [33]
		Yes	Algeria	Lardjam-Hetraf et al.,2015 [53]
SCNN1B	rs149868979	Yes	South Africa	Jones et al., 2012 [58]
SCNN1B	rs149868979	Yes	South Africa	Jones et al., 2012 [58]
FGF5	rs1458038	Yes	Uganda	Kayima et al., 2017 [33]
		Yes	Algeria	Lardjam-Hetraf et al.,2015 [53]

EBF1	rs11953630	Yes	Uganda	Kayima et al., 2017 [33]
		Yes	Algeria	Lardjam-Hetraf et al.,2015 [53]
STK39	rs3754777	Yes	Burkina Faso	Sombie et al., 2019 [3]
APOA5	rs662799	Yes	Morocco	Ouatou et al., 2014 [60]
B2	B_2 C-58T	Yes	South Africa	Moholisa et al., 2013 [63]
CYP11B2	rs1799998	Yes	Tunisia	Saidi et al., 2010 [64]
NPPA	rs748566461 C1364A	Yes	South Africa	Nkeh et al., 2002 [32]
CYP2C8	rs10509681	Yes	Ghana	Williams et al., 2004 [43]
LEP	rs7799039	Yes	Tunisia	Ben et al., 2008 [65]
SUB1	rs7726475 rs11837544	Yes	Uganda	Kayima et al., 2017 [33]
CPS1	rs1047891	Yes	Ghana	Williams et al., 2004 [43]
MOV10	rs2932538	Yes	Algeria	Lardjam-Hetraf et al., 2015 [53]

2.6 Problem statement

African populations are characterized by high genetic diversity and low levels of linkage disequilibrium among loci when compared to populations from other countries. For this reason, there are ongoing studies to understand genomic diversity within African populations. Available studies suggest that from a genetic standpoint, there is no SNPdata base that can be used to treat HTN in an African population [137]. Disease patterns in the African population call for a deeper understanding of various pathways being implicated in their varied drug response. Inferring that the lack of pharmacogenetic data serves as a hurdle to implementing tailored treatment and this may have important medical implications considering the genetic diversity of the African population. Providing an accurate genetic database on which to support future disease research in South Africa is thus of utmost importance. Consistently, population-specific SNPs are required to deliver precision medicine to all and further uncover novel disease mechanisms contributing to adverse effects of drugs. Although personalised medicine is promising to improve the individualisation of drug treatment in clinical practice [36], precision medicine research is still in its infancy because of the scarcity of genetic data on BP/HTN among the African population in South Africa [137]. The aim of the study is to investigate their inter-individual genetic variations and the risk of developing HTN in a Xhosa South African population.

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CHAPTER THREE

Systematic Review

The manuscript presented in this chapter sought to systematically extract and discuss African evidence on the effect of genetic variations in hypertensive patients and perform an *in-silico* evaluation. The purpose of this chapter is to enlighten and conform the scarcity of genetic data in an African continent and identify novel drug targets using hypertension-related genes in an African-based population. This will allow for the development of more effective HTN drug targets that could decrease the prevalence of both controlled and uncontrolled HTN. This manuscript was designed to address objective two and three.

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My contributions:

Conducted systematic literature search

Data collection

Data analysis

Wrote the manuscript

CHAPTER 3: Hypertension in African Populations: Systematic Review and Computational Insights

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Abstract

Background

Hypertension (HTN) is a persistent public health problem affecting approximately 1.3 billion individuals globally. Treatment-resistant hypertension (TRH) is defined as high blood pressure (BP) in a hypertensive patient that remains above goal despite use of ≥3 antihypertensive agents of different classes including a diuretic. Despite a plethora of treatment options available, only 31.0% of individuals have their HTN controlled. Inter-individual genetic variability to drug response might explain this disappointing outcome because of genetic polymorphisms. Additionally, the poor knowledge of pathophysiological mechanisms underlying hypertensive disease and the long-term interaction of antihypertensive drugs with blood pressure control mechanisms further aggravates the problem. Furthermore, in Africa, there is a paucity of pharmacogenomic data on the treatment of resistant HTN. Therefore, identification of genetic signals having the potential to predict the response of a drug for a given individual in an African population has been the subject of intensive investigation.

Methods

In this review, we aim to systematically extract and discuss African evidence on the genetic variation, and pharmacogenomics towards the treatment of HTN. Furthermore, *in silico* methods are utilised to elucidate biological processes that will aid in identifying novel drug targets for the treatment of resistant HTN in an African population. To provide an expanded view of genetic variants associated with the development of HTN, this study was performed using publicly available databases such as PubMed, Scopus, Web of Science, African Journal Online, PharmGKB searching for relevant papers between 1984 and 2020.

Results

A total of 2784 articles were reviewed, and only 42 studies were included following the inclusion criteria. Twenty studies reported associations with HTN and genes such as *AGT* (rs699), *ACE* (rs1799752), *NOS3* (rs1799983), *MTHFR* (rs1801133), *AGTR1* (rs5186), while twenty-two studies did not show any association within the African population. Thereafter, an *in silico* predictive approach was utilised to identify several genes including *CLCNKB*, *CYPB11B2*, *SH2B2*, *STK9*, and *TBX5* which may act as potential drug targets because they are involved in pathways known to influence blood pressure. Next, co-expressed genes were identified as they are controlled by the same transcriptional regulatory program and may potentially be more effective as multiple drug targets in the treatment regimens for HTN. Genes belonging to the co-expressed gene cluster, *ACE*, *AGT*, *AGTR1*, *AGT*R2, and *NOS3* as well as *CSK* and *ADRG1*

showed enrichment of G-protein-coupled receptor activity, the classical targets of drug discovery, which mediate cellular signaling processes.

Conclusion

The latter is of importance, as the targeting of co-regulatory gene clusters will allow for the development of more effective HTN drug targets that could decrease the prevalence of both controlled and TRH.

3.1 Introduction

Hypertension (HTN) also known as high blood pressure (BP) can be classified into primary or secondary HTN with secondary HTN affecting 5-10% of hypertensive patients with the cause linked to an underlying medical condition. In contrast, primary HTN also known as essential HTN accounts for 90-95% of HTN cases, it has no identifiable secondary root and is the most common cause of stroke and cardiovascular disease (CVD) [1, 2]. Accumulative evidence showed that the prevalence of HTN is 1.3 billion, and this number is projected to increase to 1.56 billion by 2030 with an estimated global economic cost of $274 billion [1-4]. Despite the availability of advanced diagnostic options and use of multiple antihypertensive medications, many studies have reported inadequate control of blood pressure among hypertensive individuals worldwide, including Africa [2, 5-7]. This inability to control blood pressure even after adherence has been referred to as treatment resistant HTN (TRH). According to American heart association, TRH is defined as a BP which remains ≥140/90mg despite the use of three or more antihypertensive drugs [8, 9].

Although poor control of high blood pressure may have many causes, the most likely contributor is the poor response to the prescribed antihypertensive drug [10]. Current drugs are used to treat HTN without an in-depth understanding of the biological basis that regulates the disease or the effect of an individual's make-up on the efficacy of the drug to control HTN [11]. The most commonly prescribed drug classes to treat HTN include diuretics and vasodilators designed to reduce vascular resistance. The major disadvantages are side effects (i.e. renal dysfunction) and unpredicted blood pressure responses in patients (i.e. HTN) [12, 13]. Drug classes developed from the knowledge of biological pathways include the angiotensin II antagonists and angiotensin-converting enzyme inhibitors. These have been developed through the understanding of the renin-angiotensin-aldosterone system (RAAS) in the regulation of blood pressure. As such, first-line therapy includes angiotensin-converting enzyme (*ACE*) inhibitors, angiotensin II receptors and calcium-channel blockers [14, 15]. These have proven to be the most effective drug classes (coupled with lower side effects) when used as a combination therapy [16]. Nonetheless, though effective, the one-drug- fits-all model has been proven to be ineffective due to an individual's genetic make-up and a variable

response to several antihypertensive drugs could be attributed to the genetic variability in the candidate BP regulating genes and their pathways [17]. Many studies have demonstrated that genetic factors are responsible not only for blood pressure elevation but also play a profound role in the inter-individual variability in drug response; offering an opportunity for pharmacogenomic investigation and potential individualised drug therapy [18, 19]. Therefore, it has been speculated that a patient's inter-individual genetic variation can be used to understand the pathophysiology of the disease and that this pharmacogenomics approach may have the potential of individualizing HTN drug therapy based on the patient's genetic background [20]. For example, Guo *et al*., [21] and Choi *et al*., [22] have reported successful examples of genetic polymorphisms on blood pressure response to antihypertensive therapies. These studies have highlighted the importance of understanding the biological basis of the disease and the contribution of genetic variations in the development and progression of the disease.

Despite major advances being made over the last decade, understanding the role of genetics governing the phenotypic state of HTN is complex because on one hand there rare monogenic hypertensive syndromes while on the other side are the cases of primary or TRH which may occur due to a varied expression and interactions of multiple genes [23]. However, polygenic inheritance patterns in such cases are not only complex but also enigmatic.

Nonetheless, various subtle genetic variations have been identified through approaches such as genome-wide association studies, and these small variations among individuals may account for genetic susceptibility to HTN. However, these single nucleotide polymorphisms (SNP) have been shown to vary across racial and ethnic groups and play a pivotal role during the development and progression of this phenotypic state [24]. SNPs are changes in specific nucleotides at fixed positions in DNA sequence and occur frequently once in every 1000 base pairs. These are the most commonly occurring genetic variations causing varied inter-individual drug response which remains a major public health concern [1, 3, 25]. Furthermore, it has been speculated that variable response to drugs could also be due to different subtypes of a disease phenotype in certain individuals. To exemplify, patients demonstrating better BP response with certain drugs may have different pathophysiology of HTN than others showing poor response with those drugs [18]. Therefore, a thorough understanding of the genetic background of HTN is critical to predict an individual's disease risk as well as to improve individualised treatment response. Despite the majority of SNPs having no clinical significance, several key SNPs have been reported with a detailed analysis through pharmacogenomics-based studies playing an important role in antihypertensive drug response [24, 26].

As such, the purpose of this review was to systematically extract and discuss African evidence on the effect of genetic variations in hypertensive patients and use *in silico* methods to further elucidate biological processes that will aid in identifying novel drug targets for the treatment of HTN in an African population.

3.2 Materials and Methods

A systematic search was performed using subject headings or primary search terms such as uncontrolled HTN, genetics, SNP and pharmacogenomic under the Preferred Reporting Items for Systematic Review (PRISMA) guidelines [27]. This was done using major search engines and databases such as PubMed, Scopus, Web of Science, African Journal Online and PharmGKB were used for the search as shown in Table *1S*.

3.2.1 Inclusion Criteria and Data Extraction

The studies were included in the systemic review based on the following criteria: (i) Investigated the association between SNPs and HTN in African-based population, (ii) Used case-control design, (iii) Published from 1984 to 2020, (iv) Studies done on humans (Table 3.1). Studies were excluded if duplicate publications reported selectively on migrant Africans outside Africa, family studies, used linkage analysis and analyzed mixed populations of African descent without considering their country of residence. Moreover, language restriction was applied with studies conducted in languages other than English were excluded. The articles were independently assessed for compliance with the inclusion or exclusion criteria by two authors resolving disagreements and reached a consistent decision. The following information was extracted from each study: first author and year of publication; country of origin; ethnicity of the study population; the number of subjects under hypertensive cases and controls; diagnostic criteria for hypertensive cases and controls and SNPs analyzed.

Table 3.1. Inclusion Criteria and Data Extraction.

Inclusion	Exclusion
African population	Studies in non-African countries
Published from 1984 to 2020	Studies conducted before 1983
Human studies	Non-human studies
Studies reporting an association between SNP and HTN	Studies in gene expression
Case-control design	Reviews
	Family-based studies

3.2.2 Drug-Gene Interaction

The bioinformatics tool iCTNET from Cytoscape (available at http://apps.cytoscape.org/ apps/iCTNET) was used to search through the DrugBank (available at https://www.drugbank.ca) and comparative toxicogenomic database (CTD) (available at http://ctdbase.org/) to identify drug-gene interactions. These were coupled with the occurring frequent and infrequent side-effects for each drug and plotted in a network. This analysis helped to identify the common drug-targets of HTN.

3.2.3 Co-expression networks

Networks of co-expressed genes were generated from mathematical models which were used to predict protein-protein interactions (PPIs) using the bioinformatics tool Expression Correlation from Cytoscape [28] (available at http://apps.cytoscape.org/apps/Expression Correlation). The reference database Expression Correlation utilises sample data from the human gene atlas project (available at https://www.pnas.org/content/101/16/6062) from 158 expression experiments on healthy humans. From the input gene list, Expression Correlation computes a similarity matrix using expression levels from the reference database. Gene-gene correlation networks are then computed and visualised as an interaction network.

Biological function was related to the identified co-expressed gene clusters using the gene ontology (GO) database for molecular function. This allowed for the selection of specific gene-clusters that might pose as drug targets.

3.2.4 Biological Functional Enrichment

To perform biological functional enrichment, the R Bioconductor tool Cluster Profiler [29] was used to identify enriched GO, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and disease ontologies. Fisher's exact test was performed to identify significantly enriched terms with a Benjamin-Hochberg adjusted probability value (BH adjusted p-value) of less than 0.05. This was conducted to relate biological function to the gene list related to HTN. Selecting genes as potential drug-targets with minimal influence on other biological pathways and processes was imperative to minimizing side effects in HTN patients.

3.2.5 Disease Ontology Enrichment

To identify and link the disease state to known HTN genes, disease ontology enrichment was performed using Cluster Profiler [29]. Disease ontology enrichment involved cross-referencing mappings to the Medical Subject Headings (MeSH) (available at https://www.nlm.nih.gov/databases), International Classification of Disease (ICD) (available at https://www.cdc.gov/nchs/icd/), National Cancer Institute (NCI), (available at https://www.cancer.gov/research/resources), Systematized Nomenclature of Medicine

- Clinical Terms (SNOMED CT) (available at http://www.snomed.org) and Online Mendelian Inheritance in Man (OMIM) (available at https://www.omim.org) databases. A hypergeometric model assessed the over-representation of the selected genes and their association with a disease. A BH adjusted p-value was calculated to identify significantly associated diseases (BH p-value less than 0.05). This was performed to further identify possible HTN genes that show no association with other diseases. This was a possible indicator that the targeting chosen genes would have minimal interference with other biological functions.

3.3 Results

3.3.1 Selected studies

We identified 2,784 studies through data searches: PubMed (*n* = 410), Web of Science (*n* = 1,115), Scopus (*n* = 1,245), PharmGKB (*n* = 11) and African Journal Online (*n* = 3) (Figure 3.1). After removing duplicates (*n* = 1,222) and studies that were conducted before molecular biology techniques (*n* = 43) the full text of 1,519 publications were tested for suitability [27]. Of these, 1,347 were excluded as follows; non-human studies (*n* = 29), non-HTN studies (*n* = 1,194), non-African countries (*n* = 78), reviews (*n* = 46) and different study design (e.g., investigated the expression of genes in patients with HTN, clinical studies on HTN and family-based studies) (*n* = 130). Finally, 42 studies were included in this review.

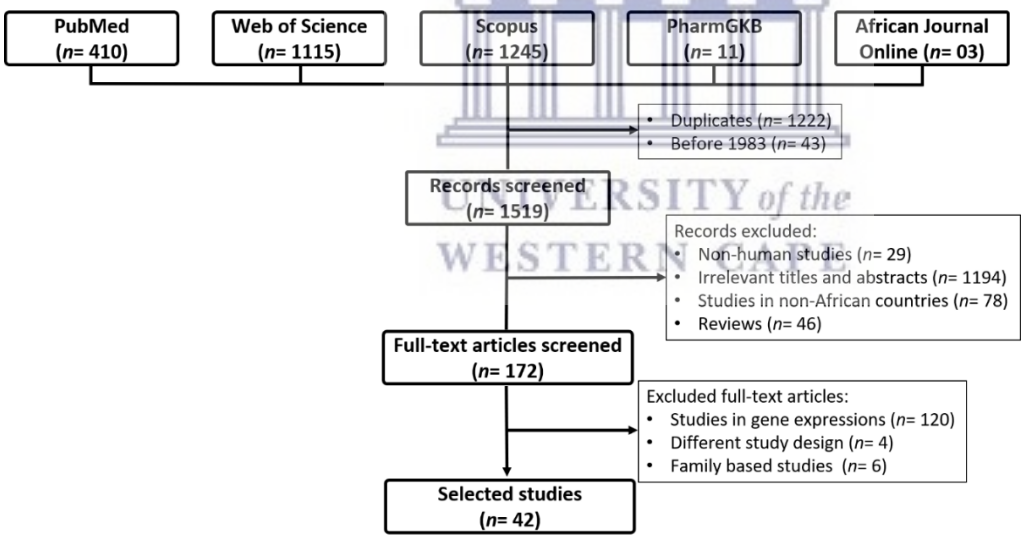

Figure 3.1 Flowchart for the study selection.

The baseline characteristics of the included studies are summarized in Table 3.1. HTN was defined as systolic/diastolic BP (SBP/DBP) ≥140/90 mm Hg or the use of antihypertensive medications at inclusion [30, 31]. Other studies used auscultatory DBP >90 mm Hg or 24-hour ambulatory DBP >85 mm Hg and auscultatory DBP >95 mm Hg, SBP/DBP >159/80 mm Hg, SBP/DBP >139/89 mm Hg and SBP/DBP

≥125/80 mm Hg,25. This review did not identify genome-wide association study (GWAS) conducted among African population with HTN.

Table 3.2 Hypertension and SNPs association in Africa

Gene	Chr	Position SNP	Alleles	Alt Allele Freq, Global (db. SNP)	Alt Allele Freq, African (db. SNP)	Cases No.	Controls	Association (p < 0.05)	Country	Author Year
AGT	1q42.2	rs2004776	C>G	0.410	0.487	782	2099	Yes	Uganda	Kayima *et al*., 2017 [32]
		rs4762	G>A	0.102	0.054	75	70	No	Algeria	Amrani *et al*., 2015 [33]
		rs699	A>G	0.705	0.903	202	204	No	Burkina Faso	Tchelougou *et al*., 2015 [34]
						612	612	No	Nigeria	Kooffreh *et al*., 2014 [35]
						81	178	No	Algeria	Meroufel *et al*., 2014 [36]
						110	93	No	Egypt	AbdRaboh *et al*. 2012 [37]
						39	22	No	Tunisia	ALrefai *et al*., 2010 [38]
						195	107	No	South African	Ranjith *et al*., 2004 [39]
AGTR1	3q24	rs5186	A>C	0.118	0.020	36	50	No	Cameroon	Ghogomu *et al*., 2016 [40]
						202	204	No	Burkina Faso	Tchelougou *et al*., 2015 [34]
						81	178	No	Algeria	Meroufel *et al*., 2014 [36]
						612	612	No	Nigeria	Kooffreh *et al*., 2014 [35]
						142	191	No	Tunisia	Mehri *et al*., 2012 [37]
						40	15	Yes	Egypt	Farrag *et al*., 2011 [41]
						195	107	No	South African	Ranjith *et al*., 2004 [39]
						NA	NA	Yes	Ghana	Williams *et al*., 2004 [42]

ACE	17q23.3	rs1799752	NA	NA	NA	202	204	Yes	Burkina Faso	Tchelougou *et al*., 2015 [26, 43]
						217	161	Yes	Egypt	Zawilla *et al*., 2014 [44]
						110	93	No	Egypt	AbdRaboh *et al*. 2012
						40	21	Yes	Egypt	Badr *et al*., 2012 [45]
						142	191	No	Tunisia	Mehri *et al*., 2012 [37]
						40	40	Yes	Egypt	Bessa *et al*., 2009 [46]
						195	107	No	South African	Ranjith *et al*., 2004 [39]
NOS3	7q36.1	rs1799983	T>A	0.824	0.930	77	77	Yes	Algeria	Amrani-Midoun *et al*., 2019 [47]
						145	184	Yes	Morocco	Nassereddine *et al*., 2018 [48]
						157	144	No	Sudan	Gamil *et al*., 2017 [49]
		rs2070744	C>T	0.766	0.862			No		
		intron 4 VNTR	N/A	N/A	N/A			Yes		
		rs1799983	T>A	0.824	0.930	70	30	Yes	Tunisia	ALrefai *et al*., 2016 [38]
		rs2070744	C>T	0.766	0.862	288	373	Yes	Tunisia	Jemaa *et al*., 2011 [50]
		rs1799983	NA	NA	NA	537	565	No	Tunisia	Sediri *et al*., 2010 [51]
		rs61722009	NA	NA	NA	295	395	Yes	Tunisia	Jemaa *et al*., 2009 [52]
MTHFR	1p36.3	rs1801133	G>A	0.245	0.090	82	72	No	Algeria	Amrani-Midoun *et al*., 2016 [53]
						41	38	Yes	Cameroon	Ghogomu *et al*.,2016 [54]
						101	102	Yes	Morocco	Nassereddine *et al*., 2015 [55]
						97	84	No	Egypt	Amin *et al*., 2012 [56]
ATP2B1	12q21.q23	rs2681472	A>G	0.199	0.094	180	200	Yes	Burkina Faso	Sombie *et al*., 2019 [3]
		rs17249754	G>A	0.209	0.131			Yes		

		rs2681492	T>C	0.208	0.126	782	2099	Yes	Uganda	Kayima *et al*., 2017 [32]
		rs17249754	G>A	0.209	0.131	189	598	Yes	Algeria	Lardjam-Hetraf *et al*.,2015 [54]
CLCNKB	1p36.3	rs12140311	A>T	0.098	0.214	213	545	Yes	Ghana	Sile *et al*., 2009 [57]
		rs34561376	G>A	0.082	0.142	213	545	No	Ghana	Sile *et al*., 2007 [58]
GNB3	12p13.31	rs5443	NA	NA	NA	388	425	No	Tunisia	Kabadou *et al*., 2013 [59]
		rs74837985	NA	NA	NA	40	40	Yes	Egypt	Bessa *et al*., 2009 [46]
CNNM2	10q24.32	rs11191548	T>C	0.152	0.025	782	2099	Yes	Uganda	Kayima *et al*., 2017 [32]
						189	598	Yes	Algeria	Lardjam-Hetraf *et al*.,2015 [54]
PLEKHA7	11p15.2	rs381815	C>A	0.206	0.190	782	2099	Yes	Uganda	Kayima *et al*., 2017 [32]
						189	598	Yes	Algeria	Lardjam-Hetraf *et al*.,2015 [54]
JAG1	20p12.2	rs1327235	A>G	0.464	0.494	782	2099	Yes	Uganda	Kayima *et al*., 2017 [32]
						189	598	Yes	Algeria	Lardjam-Hetraf *et al*.,2015 [54]
SCNN1B	16p12.2	rs149868979	NA	NA	NA	1468	471	Yes	South Africa	Jones *et al*., 2012 [60]
		rs1799979	C>T	0.007	0.024	519	514	No	South Africa	Nkeh *et al*., 2003 [61]
FGF5	4q21.21	rs1458038	C>T	0.230	0.037	782	2099	Yes	Uganda	Kayima *et al*., 2017 [32]
						189	598	Yes	Algeria	Lardjam-Hetraf *et al*.,2015 [54]
EBF1	5q33.3	rs11953630	C>A	0.07	0.180	782	2099	Yes	Uganda	Kayima *et al*., 2017 [32]
						189	598	Yes	Algeria	Lardjam-Hetraf *et al*.,2015 [54]
STK39	2q24.3	rs3754777	C>T	0.195	0.119	180	200	Yes	Burkina Faso	Sombie *et al*., 2019 [3]
APOA5	11q23.3	rs662799	G>A	0.837	0.884	149	134	Yes	Morocco	Ouatou *et al*., 2014 [62]
		rs3135506	G>A	0.056	0.067					
		rs2075291	C>A	0.011	0.002					
CDKAL1	6p22.3	rs7756992	A>G	0.413	0.633	200	208	No	Tunisia	Lasram *et al*., 2015 [63]
IGF2BP2	3q27.2	rs4402960	G>T	0.389	0.567					

TH	11p15.5	C824T	NA	NA	NA	200	202	No	South Africa	van Deventer *et al*., 2013 [64]
B2	14q32.1-q32.2	B_2 C-58T B_2 -9/+9	NA NA	NA NA	NA NA	88	77	Yes	South Africa	Moholisa *et al*., 2013 [65]
CYP11B2	8q24.3	rs1799998	A>G	0.347	0.189	537	565	Yes	Tunisia	Saidi *et al*., 2010 [66]
NPPA	1p12	rs748566461 C1364A C55A	NA	NA	NA	298	278	Yes	South Africa	Nkeh *et al*., 2002 [31]
CYP2C8	10q23.331	rs10509681 rs11572080	T>C C>T	0.046 0.046	0.008 0.008	NA	NA	Yes	Ghana	Williams *et al*., 2004 [42]
LEP	7q32.1	rs7799039	G>A	0.402	0.032	45	53	Yes	Tunisia	Ben *et al*., 2008 [67]
ADD1	4p16.3	rs4961	G>T	0.208	0.049	148	94	No	South Africa	Barlassina *et al*., 2000 [68]
ADRB2	5q32	rs1042713 rs1042714	G>A G>C	0.476 0.796	0.520 0.864	192	123	No	South Africa	Candy *et al*., 2000 [69]
SUB1	5p13.3	rs7726475	G>A	0.187	0.024	782	2099	Yes	Uganda	Kayima *et al*., 2017 [32]
CEP83	12q22	rs11837544	T>A	0.081	0.216					
IGFBP3	7p12.3	rs11977526	G>A	0.442	0.326					
CHIC2	4q12	rs11725861	A>G	0.169	0.181					
AGTR2	Xq23	rs11091046	NA	NA	NA	382	403	No	Tunisia	Kabadou *et al*., 2012 [70]
CPS1	2q34	rs1047891	C>A	0.289	0.368	NA	NA	Yes	Ghana	Williams *et al*., 2004 [42]
MOV10	1p13.2	rs2932538	A>C	0.830	0.842	189	598	Yes	Algeria	Lardjam-Hetraf *et al*., 2015 [54]
SLC4A7	3p24.1	rs13082711	T>C	0.120	0.036					
MECOM	3q26.2	rs419076	T>A	0.584	0.450					
SLC39A8	4q24	rs13107325	C>A	0.024	0.002					
GUCY1A1N	4q32.1	rs13139571	C>A	0.211	0.129					
PR3	19p13.3	rs1173771	A>G	0.661	0.795					
HFE	6p22.2	rs1799945	C>G	0.073	0.011					
BAG6	6p21.33	rs805303	G>A	0.436	0.643					
CACNB2	10p12.33	rs4373814	G>C	0.512	0.613					
PLCE1	10q23.33	rs932764	A>G	0.428	0.184					
CAND1	12q14	rs7129220	G>A	0.058	0.052					
ARHGAP42	11q22.1	rs633185	G>A	0.639	0.804					
FES	15q26.1	rs2521501	A>C	0.213	0.223					
GOSR2	17q21.32	rs17608766	T>C	0.054	0.010					
ZNF831	20q13.32	rs6015450	A>G	0.098	0.205					
ULK4	3p22.1	rs3774372	T>C	0.173	0.197					
CABCOCO1	10q21.2	rs4590817	G>C	0.110	0.190					

SH2B3	12q24.12	rs3184504	T>A	0.853	0.981
TBX5	12q24.21	rs10850411	T>C	0.470	0.346
CSK	15q24.1	rs1378942	C>A	0.245	0.026
ZNF652	17q21.32	rs12940887	C>T	0.185	0.048

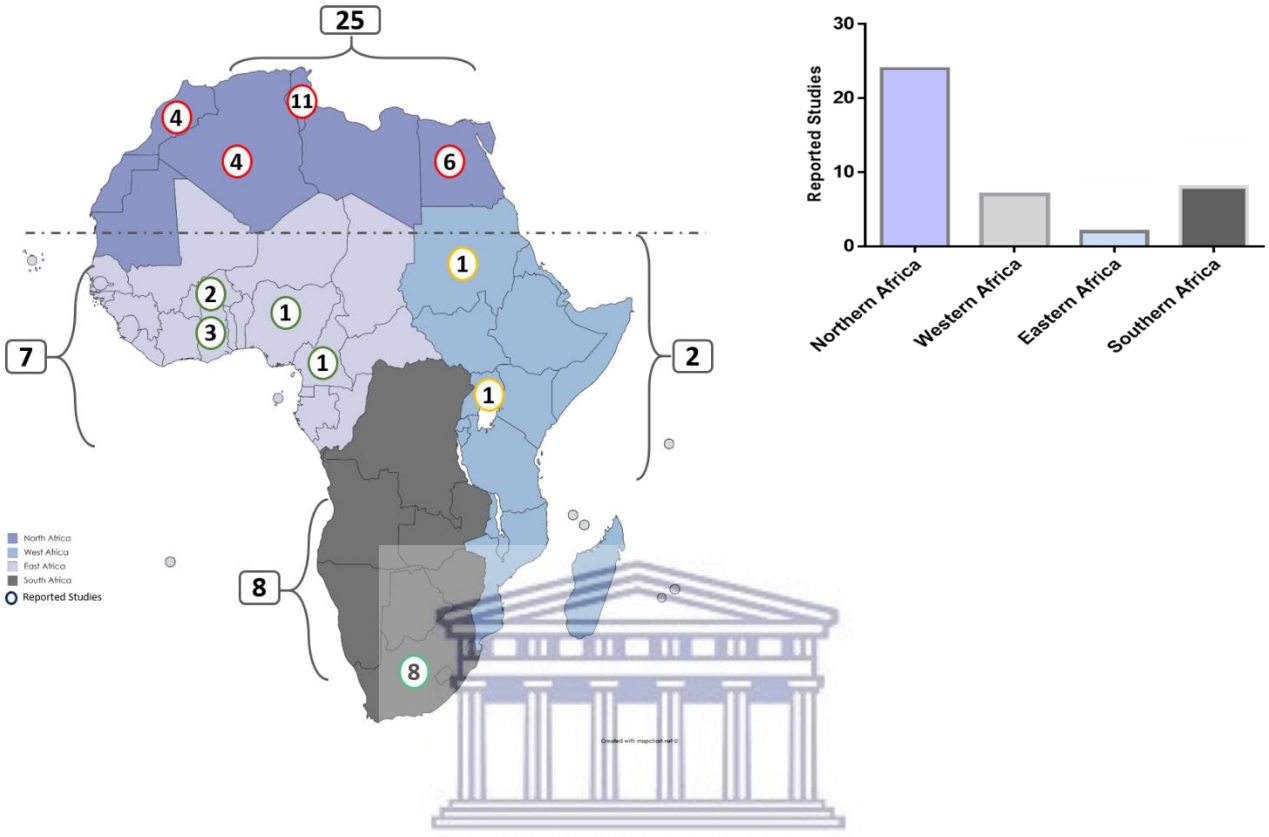

Figure 3.2 Summary of all genetic studies reported in the African continent in relation to hypertension.

Northern African countries (*n* = 25) appeared to have more studies carried on HTN and SNPs association sub-Saharan African countries (Eastern Africa = 2, Western Africa = 7, Southern Africa = 8) as elaborated in Figure 3.2. Twenty studies reported an association between HTN and genes such as *ACE, AGT, AGTR1, ANP, APOA5, ARGHGAP42, ATP2B1, B2, BAG6, CABCOCO1, CACNB2, CAND1, CHIC2, CNNM2, CPS1, CSK, CYP11B2, CYP2C8, EBF1, FES, FGF5, GNB3, GOSR2, GRK4, GUCY1A1N, HFE, IGFBP3, JAG1, LEP, MECOM, MOV10, MTHFR, NOS3, PLCE1, PLEKHA7, PR3, SH2B3, SLC39A8, SLC4A7, STK39, SUB1, TBX5, ULK4, ZNF652*, and *ZNF831* whereas twenty-two studies did not show any association (Table 3.2). The most studied genes were *AGT*, *ACE*, *NOS3*, *AGTR1*, *MTHFR*, *ATP2B1*, *CYP2C8*, *GNB3*, *CNNM2*, *PLEKHA7*, *JAG1*, *FGF5* and *EBF1* as sequentially arranged from highest to the smallest number of studies (see Table 3.2). Other genes reported in single studies were *STK39, CDKAL1, IGF2BP2, TH, B2, CYP11B2, LEP, CLCNKB, SCNN1B, ADD1, ADRB2, SUB1, CEP83, IGFBP3, CHIC2,*

AGTR2, CPS1, MOV10, SLC4A7, MECOM, SLC39A8, GUCY1A1N, PR3, HFE, BAG6, CACNB2, PLCE1, CAND1, ARHGAP42, FES, GOSR2, ZNF831, ULK4, CABCOCO1, SH2B3, TBX5, CSK and *ZNF652*.

3.3.2 Gene-Drug Interaction and Ontology Analysis

A gene list consisting of 53 genes linked to HTN was analyzed to gain an understanding of drug interaction, biological processes, pathways and various associated diseases Food and Drug Administration (FDA) approved drug list was obtained and used to identify drug interactions with the above-mentioned genes. Identification of potential drug-gene interactions is the first step in a translation research effort aiming to reduce the burden of a major public health problem such as CVD **[71]**. Therefore, in the current study Drug Bank and CTD databases were utilised to generate a drug-gene interaction network (Figure 3.3) that identified the genes interacting with FDA approved HTN drugs. Of the 53 genes, only 14 genes (*CYP2C8, CYP11B2, AGT, AGTR1, AGTR2, ACE, ADRB2, LEP, MTHFR, NOS3, HFE, CNNM3, IGF2BP2* and *SCNN1B*) showed an interaction with marketed drugs along with their corresponding side-effects (Figure 3.3). GO enrichment analysis was performed to identify the enriched biological processes that the gene list regulates (Figure 3.4). Finding ontologies linked to biological processes provides insights into the underlying mechanisms regulated by the chosen gene list. This allows for the identification of additional drugs that have previously not treated HTN.

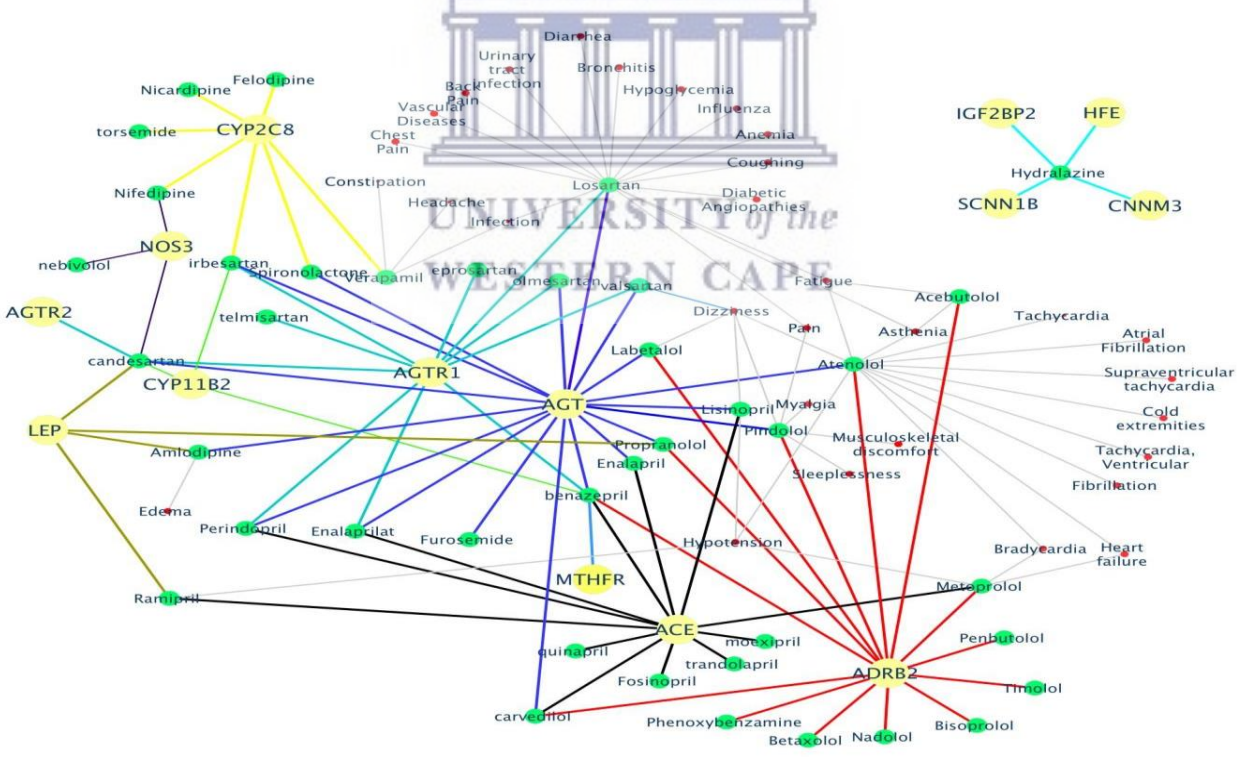

Figure 3.3 The identified drug-gene interactions along with the side-effects are plotted into a network. Only 14 out of the total 53 HTN genes (shown as yellow nodes) mapped to 57 FDA approved HTN drugs (shown as green

nodes) and their corresponding side-effects (shown as red nodes). The edges in the drug-gene interaction network are shown in a different colour for each gene to easily distinguish their association with the various HTN drugs.

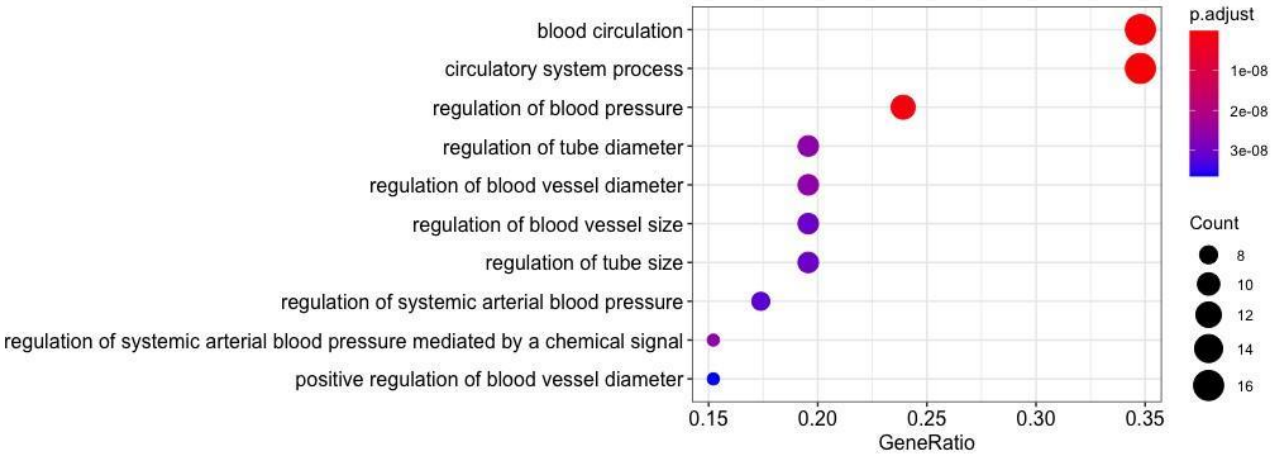

Figure 3.4 The gene ontology analysis performed on all 53 prioritised HTN related genes to identify related biological processes. The gene ratio of participating genes in the enriched ontology, the colour-coded BH-adjusted p-value, and the number of genes (count) in each enriched ontology are shown in the above plot.

Co-expression analysis was performed to identify co-expressed gene clusters (Figure 3.5). These were interrogated further to relate the molecular function for each gene cluster (Figure 3.6). This was performed to identify potential mechanisms in which drugs can be targeted against HTN. Genes in co-expressed gene clusters and their linked HTN drugs are summarized to explore the potential combination therapy options for HTN.

3.3.3 Co-expression Network

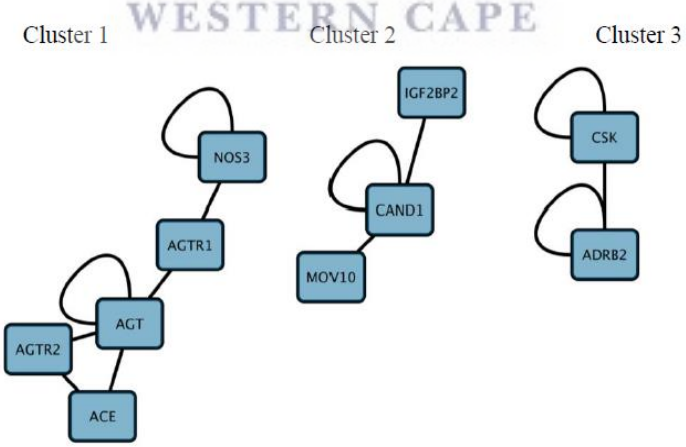

Figure 3.5 Co-expression networks generated to identify co-regulated gene clusters from the 53 prioritised genes related to Hypertension Co-expressed gene clusters have been numbered above from 1 to 3.

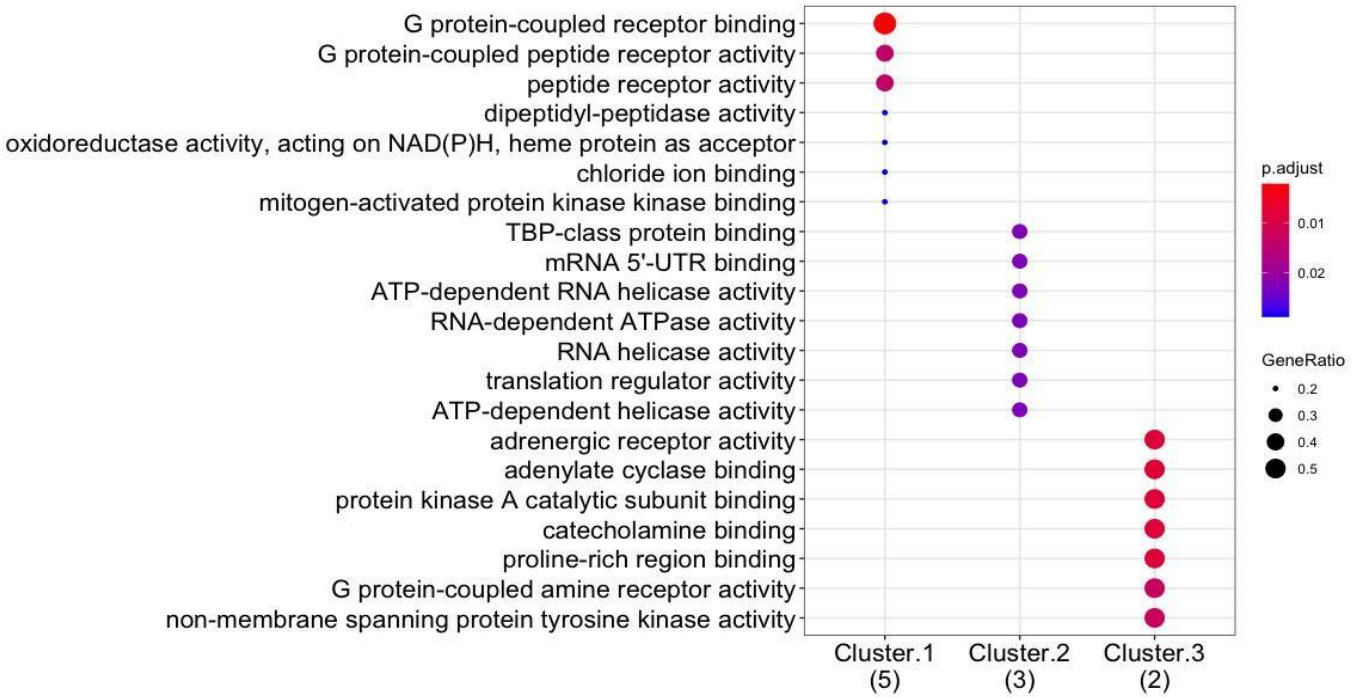

Figure 3.6 GO enrichment (i.e., molecular function) performed on the co-expressed gene clusters (labelled cluster 1-3, and the number of genes in brackets) to relate molecular function to co-expressed genes. The gene ratio of participating genes in the enriched ontology, and the colour coded BH-adjusted p-value are shown in the plot.

3.3.4 Co-expression Ontology Analysis

Similar to the GO enrichment analysis performed, KEGG pathway analysis (Figure 3.7) was performed to identify the enriched pathways regulated by the 53 HTN genes. This is performed to identify alternative pathways that interact with HTN related pathways, which can provide insights into how HTN drugs may influence various biological pathways.

3.3.5 Biological Pathway Analysis

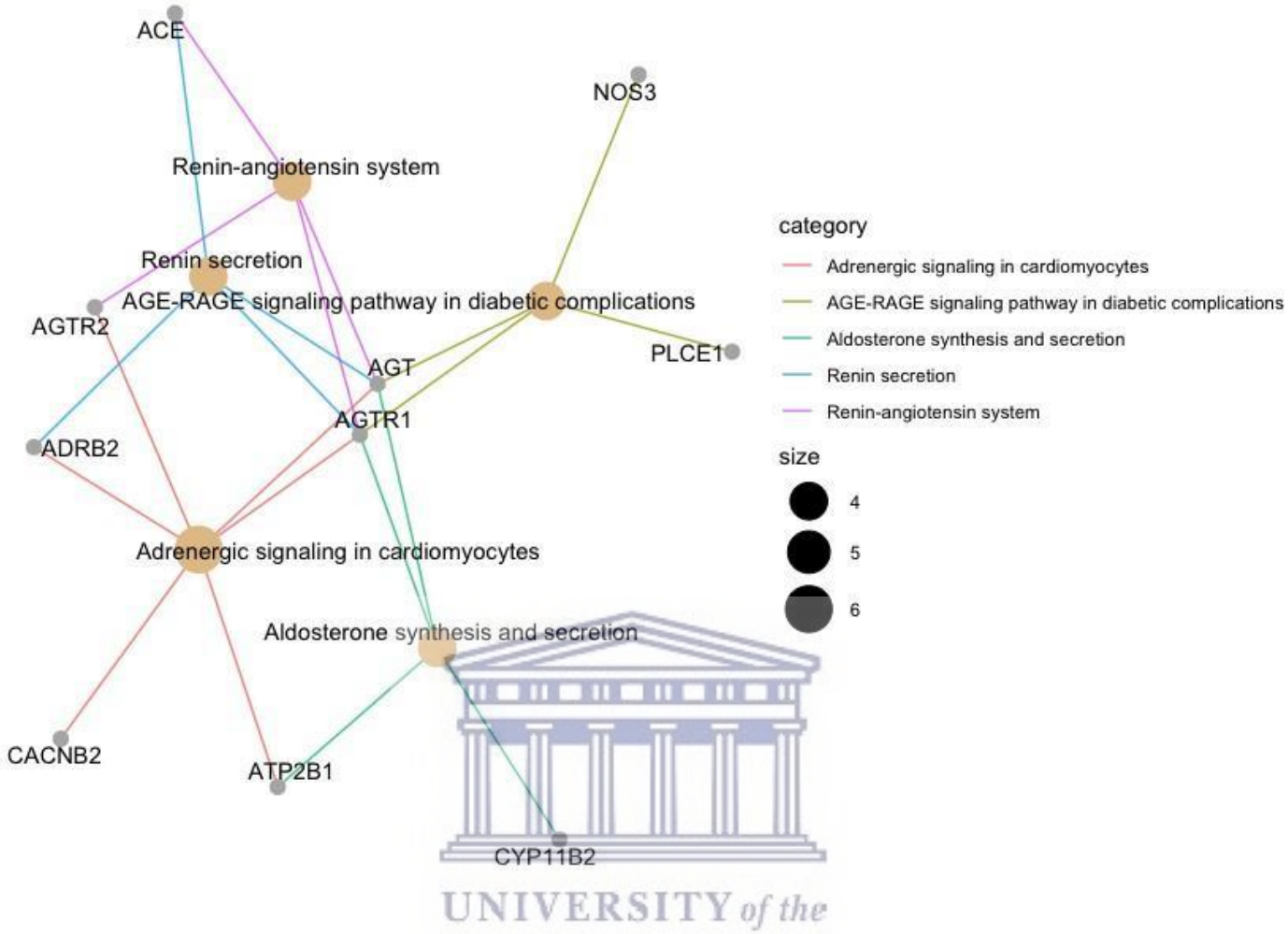

Figure 3.7 Pathway enrichment analysis performed on the 53 HTN genes annotated using the KEGG database. Only 10 from the 53 HTN genes mapped to the KEGG database linking them to biological pathways. The plot illustrates the participating genes with the enriched pathway. Each enriched pathway has been colour-coded, and the number of participating genes corresponds to the size of the node.

Disease ontology (Figure 3.8) was also performed to analyze the involvement of the 53 HTN genes in various other diseases. This analysis gives insights into how different diseases may share common gene expression patterns and how drug treatment influences several biological pathways causing undesired side effects. In the selection process of identifying genes that are only linked to HTN and other related diseases, disease ontology enrichment analysis was conducted.

3.3.6 Disease Ontology

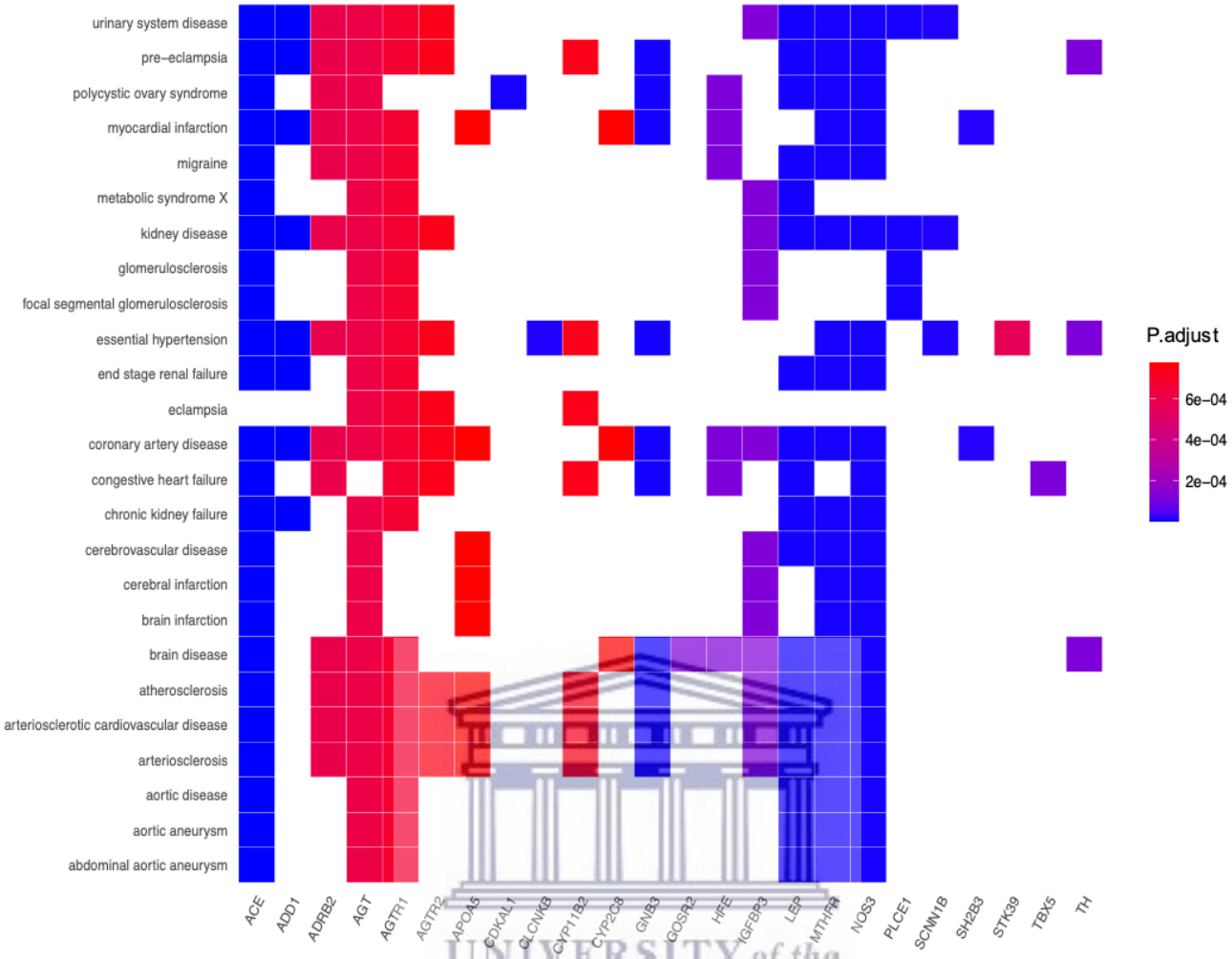

Figure 3.8 The enrichment of disease ontology for the mapped genes related to hypertension. Each gene is statistically mapped to a disease. The matched boxes associated with the enriched disease term are colour coded by the BH-adjusted p-values.

Disease ontology built from a disease-gene interaction database was plotted in a network (Figure 3.9) to visualise the interactions between genes and enriched diseases. This graph serves to identify genes that are targeted in HTN treatment however are also linked with other diseases.

3.3.7 Gene-Disease Association

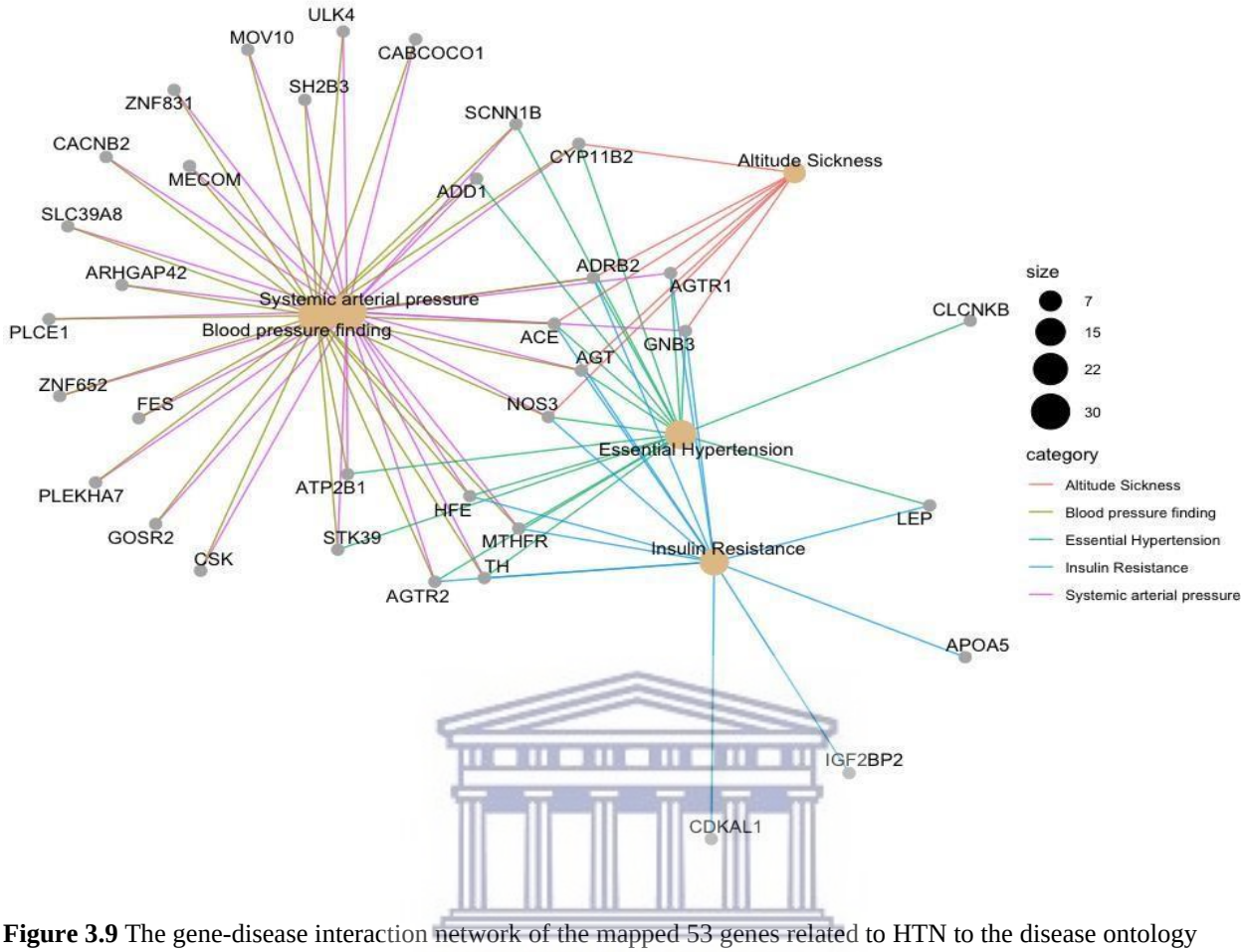

Figure 3.9 The gene-disease interaction network of the mapped 53 genes related to HTN to the disease ontology database. The size of the enriched disease illustrated as a node corresponds to the number of participating genes. The colour of the edges corresponds to the enriched disease.

3.4 Discussion

HTN is a multifactorial disease affecting one billion individuals. It is a leading cardiovascular risk factor accounting for premature deaths and has a significant economic cost [15]. Despite the availability of many antihypertensive drugs, less than 50% of patients have their blood pressure controlled [72]. These disappointing outcomes could be because of medication non-adherence and/or inter-individual variation [26, 73]. Considering studies linking inter-individual variation to drug response, several studies have tested SNPs as predictors of RHT risk [74-76]. Various studies have reported an association between SNPs and HTN in European [77, 78], African American [79] and Asian population [79-81], however not much evidence has been got on the association of specific SNP or its associated haplotypes with HTN in an African population [26]. Therefore, we systematically extracted and discussed evidence on the African genetic variation and pharmacogenomics towards the treatment of HTN.

A total of 42 studies comprising of 53 genes are included in this review, however, only 20 studies reported a significant association with the risk of HTN. Table 3.2 highlighted candidate genes such as *ACE, NOS3, ATP2B1* and *MTHFR* that have repeatedly been implicated to have an association with HTN in various African populations.

3.4.1 Angiotensin-converting enzyme gene

Angiotensin-converting enzyme (*ACE*) is a metalloenzyme that cleaves angiotensin I to angiotensin II and inactivates a potent vasodilator (bradykinin) [82]. *ACE* is encoded by the *ACE* gene which has been mapped to chromosome 17q23, and it has 26 exons and 25 introns [83]. Single nucleotide polymorphism (rs1799752) on the *ACE* gene was investigated for its implication on high blood pressure in different sub-Saharan Africa countries (Table 3.2). It has been showed in various studies conducted in different parts of Africa continent that rs1799752 could be a potential genetic predictor for the development of HTN and permit initiation of personalised medicine [76,77,78]. However, other studies performed in South Africa, Tunisia, and Egypt exhibited no association of *ACE* gene polymorphism with the risk of developing HTN [37, 84, 85].

3.4.2 Nitric oxide synthase gene

Nitric oxide synthase (NOS3) gene is another important candidate gene because of its critical role in regulating blood pressure. This gene (NOS3) is on chromosome 7q35-36, comprising 26 exons. *NOS3* is an integral component of vasorelaxing pathway, which is mediated by endothelium derived nitric oxide [86]. Various studies summarized in this review showed that different populations from Africa exhibit positive association of genetic variants of *NOS3* such as rs1799983 [47], rs2070744 [50], rs149868979 [60] and rs61722009 [87] with the risk of developing HTN. However, two studies in this review that aimed to investigate the *NOS3* gene variants associated with the development of HTN showed no association with the disease [49, 51].

3.4.3 Plasma membrane calcium-transporting ATPase 1 gene

In 2009, the genome-wide association studies (GWAS) have identified the association between the Plasma membrane calcium-transporting ATPase 1 (*ATP2B1*) polymorphisms (rs2681472, rs17249754, rs2681492) and HTN in diverse populations [88, 89]. This gene encodes for plasma membrane calcium dependent ATPase, which handles calcium pumping to the extracellular compartment [90]. In this review, it was revealed that genetic variants in ATP2B1 genes in some populations from Algeria, Burkina Faso and Uganda are associated with the risk of developing HTN. For instance, a study conducted in an Algerian population reported that the *ATP2B1* variant (rs17249754) has a robust significant association with HTN [91]. The findings were further replicated in the Burkinabe population [3]. In another study conducted by

Kayima *et al.* [92] on a Ugandan population, it was reported that rs2681492 was significantly associated with HTN in hypertensive individuals. Likewise, SNPs on *ATP2B1* (rs17249754, rs2681472 and rs2681492) were reported to be in linkage disequilibrium and located in the same linkage disequilibrium block in the Chinese population [93].

3.4.4 Methylenetetrahydrofolate reductase gene

Methylenetetrahydrofolate reductase gene (*MTHFR*) is one of the most studied genes associated with HTN in the African countries. *MTHFR* is on chromosome 1p36.3 and is well known to be involved in the metabolism of homocysteine and folate [94]. Previous studies have shown that the *MTHFR* variant such as rs1801133 has been associated with HTN between 24-87% [95-97]. Similarly, in this review, 50% of studies investigating the role of *MTHFR* provided a positive line of evidence linking this gene with HTN, showing that the rs1801133 polymorphism in *MTHFR* increases the risk of HTN [91, 98, 99]. Conversely, Amrani-Midoun *et al.* [47] and Amin *et al*. [56] in Algeria and Egypt showed no association of rs1801133 in hypertensive patients.

Furthermore, genes such as *CYP11B2* (rs1799998) [66], *LEP* (rs7799039) [67] and *CPS1* (rs1047891) [100] were also reported to be associated with HTN, however, these findings could not be replicated in the different African populations.

Furthermore, we attempted to shortlist key target genes out of the 53 identified genes in this review by using bioinformatics methods on gene-specific data available in various public databases. The bioinformatics analysis investigated various parameters associated with the 53 HTN genes such as drug interactions, co-expression of genes and disease ontology and linked pathways. Only 14 genes (*CYP2C8, CYP11B2, AGT, AGTR1, AGTR2, ACE, ADRB2, LEP, MTHFR, NOS3, HFE, CNNM3, IGF2BP2* and *SCNN1B*) were identified to have an interaction with FDA approved marketed HTN drugs (Figure 3.3). As evident from Figure 3.3, the various antihypertensive drugs such as losartan, acebutolol, atenolol, metoprolol, ramipril, lisinopril, labetalol and amlodipine show distinct side-effects. It has also been reported in the literature [101-103] that 20-97% of patients taking antihypertensive drugs suffer from various drug-related side effects. Furthermore, these side effects may potentially also account for non-adherence to antihypertensive drugs [104-106]. Losartan, an angiotensin II antagonist and atenolol, a beta-blocker showed the most side-effects related to respiratory impairments and infection. Prior studies have also documented a higher frequency of side-effects and lower adherence among patients taking diuretics and beta-blockers [107]. In hypertensive patients of African ancestry, diuretics and or calcium channel blockers (amlodipine) are preferably recommended [108] and patients who remain concerned about the adverse health effects of antihypertensive drugs are less likely to be adherent to their medications [109].

The co-expression analysis identified three clusters, including cluster 1 (*ACE*, *AGT*, *AGTR1*, *AGTR2* and *NOS3*), cluster 2 (*MOV10*, *CAN1* and *IGF2BP2*), and cluster 3 (*CSK* and *ADRB2*). Upon further analysis, from cluster 1, *AGTR1*, *AGTR2*, *AGT* and *ACE*; cluster 2, *IGFBP2* and cluster 3 *ADRB2* genes are shown to be mapped to HTN drugs shown in Figure 3.3. Interestingly, the protein-coding genes *AGTR1*, *AGTR2*, *AGT* and *ACE* are all involved in the RAAS cascade which regulates blood pressure and vascular resistance. As such, the most widely used HTN drug classes include *ACE* inhibitors, angiotensin II antagonists and direct renin inhibitors which target the RAAS cascade [110]. Whereas, in the second and third cluster, the genes *IGFBP2* (targeted by vasodilators) and *ADRB2* (targeted by beta-blockers), respectively, have been recently linked with increased risk of HTN and have been recently proposed as potential diagnostic biomarkers for HTN. For example, in a study by Yang *et al*. [111] the overexpression of the gene *IGFBP2* which is linked to the regulation of vascularisation and angiogenesis is associated with the increased severity of HTN [111]. Additionally, the gene *ADRB2* has been shown to interact with the gene *NOS3* [112]. *ADRB2* may indirectly interact with the RAAS cascade via the *NOS3* forming part of the gene cluster of genes associated with the RAAS cascade (Figure 3.5). This is further illustrated in Figure 3.7 where *ADRB2* shows interaction with RAAS associated genes by regulating common biological pathways including renin secretion and adrenergic signaling. Lastly, the observations in Figures 3.7-9 highlight the genes *ADRB2*, *ACE*, *AGT* and *NOS3* to have an association with the metabolic syndrome (includes HTN, type II diabetes, excess lipids and abnormal cholesterol levels). Although further validation is required, the above-mentioned genes exhibit characteristics of being key regulators in metabolic syndrome.

This is also evident from the biological processes and pathways showed enrichment of HTN (by vasoconstriction and RAAS) and diabetic-related terms (see Figure 3.6 and 3.7). *NOS3* (mapped to drug-gene interaction network) and *PLCE1* showed no interaction with HTN related *KEGG* pathways but exclusive to *AGE-RAGE* signaling pathways in diabetic complications. As such, using drugs targeted against HTN may influence other signaling pathways. In this case, *NOS3* interacts with 3 HTN drugs (Angiotensin II antagonists, calcium channel blockers and beta-blockers).

It is also important to understand the roles that genes play in different sub-types of HTN. Figure 3.8 shows that certain genes are shared among different sub-types of HTN and there are genes which contribute to a specific sub-type of the disease. For example, *CLCNKB* is only linked to TRH, therefore, a drug targeting this gene will be effective in treating salt sensitive HTN, since a polymorphism in this gene leads to increased salt retention in the bloodstream. Similarly, the gene such as *ACE* is linked to almost all categories of diseases shown in Figure 3.8, thus pointing towards its universal role in HTN related diseases.

From the disease ontology analysis, the following potential gene targets have been selected, these include *CLCNKB, CYPB11B2, SH2B2, STK9* and *TBX5* as they show interactions exclusively with HTN -related pathways (see Figure 3.8). Furthermore, targeting co-regulated gene clusters as opposed to single-gene targeting increases the likelihood of more effective drug treatment for HTN. Cluster 1 (*ACE, AGT, AGTR1, AGTR2* and *NOS3*) and cluster 3 (*CSK* and *ADRG1*) showed enrichment of G-coupled receptor binding activity (a classical target of drug discovery) and protein tyrosine kinase receptors (see Figure 3.5), thus demonstrates that these gene clusters can be targeted during future drug design. Therefore, we anticipate that these potential genes including their SNPs need to be investigated further in African populations. [113, 114]

As aforementioned, *ACE* and *NOS3* genes have been studied for its implication on HTN in more than six published papers indexed on the different databases, of which 50% of those were able to link these genes polymorphisms with the high risk for HTN. This suggests that there was a significant association of *ACE* and *NOS3* variants with HTN in African populations. However, *AGT, AGTR1* and *AGTR2* polymorphisms in African populations showed less than 25% or no association with HTN. For example, all the SNPs located near the *AGT* gene, only rs2004776 was demonstrated to have a strong association with HTN in Ugandan population [81]. Furthermore, Farrag *et al*. [41] reported the positive association of *AGTR1*, rs5186 with HTN in Egyptian population and similar findings were also observed in the Ghanaian population. However, no association has been recorded in other *AGTR1* and *AGTR2* polymorphisms.

While several studies in this review support an important contribution of genetic polymorphisms in HTN, some studies have failed to detect significant effects or replicate previous findings. These inconsistencies may be linked to the African populations high genetic diversity and low levels of linkage disequilibrium among loci when compared to populations from other countries outside Africa [115]. Furthermore, studies suggest that from a genetic standpoint, there is no SNP-database that can be used to personalise treatments for HTN in an African population [47, 116, 117]. Inferring that the lack of genetic information with robust allele frequency distributions serves as a hurdle to implement corrective treatment and this may have important medical implications [118]. Providing a more accurate reference foundation on which to support future disease research in Africa is thus of utmost importance [26].

3.5 Conclusion

In conclusion, the present systematic review report on the susceptibility of inter-individual genetic variation to HTN in African population. Large-scale genetic studies are needed to better understand the susceptibility of African population-based inter-individual genetic variation and their effect on the hypertensive drug

response, which will aid in the development of effective African based individualised antihypertensive medicine.

3.6 Acknowledgements

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3.7 Author contributions

S.E.M conducted systematic literature search and collected and analyzed the data and compiled the first draft of paper. L.M. and M.K performed bioinformatic analysis and reviewed and edited the manuscript. J.R.S. contributed to design, analysis and edited the manuscript. T.A reviewed and edited the manuscript. B.M contributed to design, analysis of the manuscript. M.B. reviewed and edited the manuscript. RJ conceptualised, designed, reviewed and edited the manuscript.

3.8 Conflicts of interest

None.

3.9 References

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CHAPTER FOUR

A Narrative Synthesis of Literature

Methylenetetrahydrofolate reductase gene (*MTHFR*) gene is well known to be interlinked with nitric oxide synthase 3 (*NOS3*) and contribute to the production of nitric oxide, a potent vasodilator regulating blood pressure. Furthermore, *MTHFR* (rs1801133) has been reported as one of the most researched genes in our previous systematic review (**chapter three**) among the hypertension-related genes, currently reported in an African population. Therefore, the purpose of this narrative synthesis of literature chapter sought to gather and analyse the available genetic evidence on the association between *MTHFR* (rs1801133) and the risk of developing hypertension in an African population and further compare the evidence with global data. This manuscript was designed to address objective four.

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My contribution:

Conceptualised and designed the study

Analysed and interpreted the data

Drafted the manuscrip

CHAPTER 4: Methylenetetrahydrofolate Reductase Polymorphism (rs1801133) and the Risk of Hypertension Among African Populations: A Narrative Synthesis of Literature

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Abstract

In this review, we have gathered and analyzed the available genetic evidence on the association between the methylenetetrahydrofolate reductase gene (*MTHFR*), rs1801133 and the risk of Hypertension (HTN) in African populations, which was further compared to the global data evidence. This review was reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol and Human Genome Epidemiology Network (HuGENet) guidelines. Literature was retrieved through major search databases, including PubMed, Scopus, Web of Science, and African Journal Online. We identified 64 potential studies, of which 4 studies were from the African continent and 60 studies were reported globally. Among the studies conducted in Africa, only two (n = 2) reported a significant association between the *MTHFR* (rs1801133) and the risk of developing HTN. Only one (n = 1) study population was purely com-posed of black Africans, while others were of other ethnicities. Among studies conducted in other continents (n = 60), forty-seven (n = 47) studies reported a positive association between *MTHFR* (rs1801133) and the risk of developing HTN, whereas the remaining studies (n = 14) did not show a significant association. Available literature suggests an apparent association between rs1801133 and HTN in global regions; however, such information is still scarce in Africa, especially in the black African population.

Keywords: Hypertension; methylenetetrahydrofolate reductase gene; *MTHFR*; single-nucleotide polymorphism; Africa; genetic variation

4.1. Introduction

Hypertension (HTN) remains a major risk factor for the development of cardiovascular diseases (CVDs), which significantly contributes to high rates of mortality and morbidity worldwide. Globally, HTN affects over 1.4 billion individuals above the age of 18 years and the number is expected to increase to 1.56 billion by 2025 [1–3]. In Africa, HTN affects approximately 74.4 million individuals [4,5]. Although there are various treatments available for HTN, it is apparent that patients are now gaining resistance to the treatment, and more severe cases have been recorded, particularly among individuals of African origin [5,6]. Furthermore, the high prevalence and severity of HTN that has been observed across different populations have been attributed to genetic variation [7]. Generally, it has been reported that genetic factors contribute to approximately 30–60% of the blood pressure (BP) variation that has been observed [8,9]. Therefore, it is critical to explore genetic factors with regards to HTN with the aim of understanding their role in the pathogenesis and progression of the disease.

Many approaches have been used to identify genetic variants associated with HTN in various populations [1,10,11]. The most common type of genetic variants are single nucleotide polymorphisms (SNPs), which represent approximately 90% of human genetic variations [12]. Several genome-wide association studies have identified multiple SNPs associated with HTN [13,14]. Amongst the predominantly identified variants is the SNP rs1801133 (position 677 C>T) found in exon 4 of the Methylenetetrahydrofolate reductase gene (*MTHFR*), which has been reported to be associated with elevated BP in various populations [15,16].

The *MTHFR* gene sits on the short arm of chromosome 1 (1p36.22), which has 12 exons and encodes for a protein containing 656 amino acids [17,18]. *MTHFR* is an enzyme that facilitates the production of 5-methyl-tetrahydrofolate, an active form of folate (Vitamin B9) in the body [19]. Previous research has demonstrated that 5-methyl-tetrahydrofolate is a positive allosteric modulator of nitric oxide synthase 3, which plays a significant role in the production of nitric oxide, a potent vasodilator in the regulation of BP [20]. Moreover, the *MTHFR* gene polymorphism has been suggested to be associated with increased levels of plasma homocysteine (hyperhomocysteinemia), which acts as an independent risk factor for HTN [21,22]. Factors like excessive alcohol consumption and smoking can influence the elevation of homocysteine in blood plasma [23,24]. According to a cross-sectional study conducted among women, the association between folate intake and homocysteine was altered by both alcohol intake and *MTHFR* rs1801133 [25].

A meta-analysis by Wu *et al.* [26] already demonstrated that *MTHFR* gene polymorphisms are linked with a significantly increased risk of HTN in subjects that carry the T allele and TT genotype. Another meta-analysis by Yang *et al.* [27], which was conducted in Indiana, United States of America, also reported an association between *MTHFR* (rs1801133) and HTN. However, this association was only significant among

Asian and Caucasian populations, while no correlation was observed for Latinos, Africans, and Indians, suggesting the implication of ethnicity in disease susceptibility. Importantly, the authors acknowledged the essential limitations, such as the relatively small sample size and data scarcity for Latinos, Africans, and Indians. Furthermore, studies conducted in Morocco [16] and China [28] suggested that *MTHFR* (rs1801133) is associated with an increased risk of HTN. Conversely, Amrani-Midoun *et al.* [15], reported no association in the Algerian population. Thus, information regarding the correlation between *MTHFR* polymorphism (rs1801133) and HTN remains elusive, especially among black Africans. This review has extracted and critically analyzed the available clinical evidence on the association of *MTHFR* (rs1801133) and HTN in African populations, and further compared the evidence with studies conducted in other parts of the world.

4.2. Methods

4.2.1. Search Strategy

A comprehensive literature search was performed using subject headings or primary search (MeSH) terms such as "Methylenetetrahydrofolate reductase gene", "*MTHFR*", "hypertension", "genetic*", "single nucleotide polymorphism", and "pharmacogenomics" following the Human Genome Epidemiology Network (HuGENet) [29,30] and PRISMA guidelines [31,32]. The reference lists of included studies were further scanned for additional relevant studies. The search was done using major search engines and databases, including PubMed, Scopus, Web of Science, and African Journal Online. However, this review was not registered with online registries; therefore, the protocol does not have a registration number. Nevertheless, the aforementioned search engines and databases were thoroughly searched to make sure no other similar studies are currently underway.

4.2.2. Inclusion Criteria and Data Extraction

Studies included in the current review meet the following requests: (a) only the case-control studies were considered; (b) evaluated the *MTHFR* gene, rs1801133 polymorphism, and HTN risk; and (c) studies with data on the genotypes among cases and controls [33]. Studies were excluded if (a) conducted before the inception of molecular biology techniques (1983), (b) non-human studies, (c) family studies, and (d) reviews (Table 4.1). The data were independently and carefully assessed for compliance with the inclusion or exclusion criteria by three authors (S.E.M, K.Z, and C.M) who resolved disagreements and reached a consistent decision with the help of a fourth investigator (B.M). The following information was extracted from each study: the first author, publication year, country, ethnicity, continent, number of cases and controls, source of controls, and Hardy-Weinberg Equilibrium (HWE). Language restriction was applied during the search meaning studies conducted in other languages that could not translate into English were excluded.

Table 4.1. Inclusion Criteria and Data Extraction.

Inclusion	Exclusion
Published from 1984 to 2021	Studies conducted before 1983
Human studies	Non-human studies
Reported data on the genotypes among cases and controls	No genotypes among cases and controls
Studies reporting association between *MTHFR* polymorphisms (rs1801133) and HTN	Studies in gene expression
Studies provided enough data to calculate ORs and 95% confidence interval	Studies provided not enough data to calculate ORs and 95% confidence interval
Case-control design	Reviews
Non-family-based studies	Family-based studies

4.3. Results

There are very limited studies reporting on *MTHFR* (rs1801133) association and HTN in African populations. Out of all identified relevant studies, only one study population was indigenous African (Cameroon) [34]; others were composed of Caucasian participants (Algeria, Morocco, Egypt) [15,16,35]. For this reason, a narrative synthesis of the findings was performed, instead of a meta-analysis.

4.3.1. Characteristics of Studies

Using our search strategy (Figure 4.1), we have identified 1230 related studies, of which four (n = 4) were from the African continent and (n = 60) were from non-African continents (globally). Based on our inclusion and exclusion criteria, there were 321 cases and 308 controls for the African population and 15,865 cases and 28,762 controls for other global populations globally, that were available for this analysis. The study characteristics are described in Table 4.1. In all the studies reported in Africa [15,16,34,35], HTN was defined as systolic/diastolic BP (SBP/DBP) ≥ 140/≥ 90 mm Hg. Among the included studies reported in the African region (n = 4), only two (n = 2) studies reported a significant association between the *MTHFR* (rs1801133) and the risk of developing HTN [16,34]. All African studies, where age was reported, included only patients aged above 40 years, except for Amin *et al.* [35], which included patients aged ≤ 45 years. Furthermore, most African studies included more females than males with exception of Amin *et al.* [35] which did not report on gender. In studies reported in other continents (n = 60), forty-seven separate studies showed a significant association between *MTHFR* (rs1801133) and the risk to develop HTN Table 4.2, whereas the remaining studies did not show any significant association (n = 14).

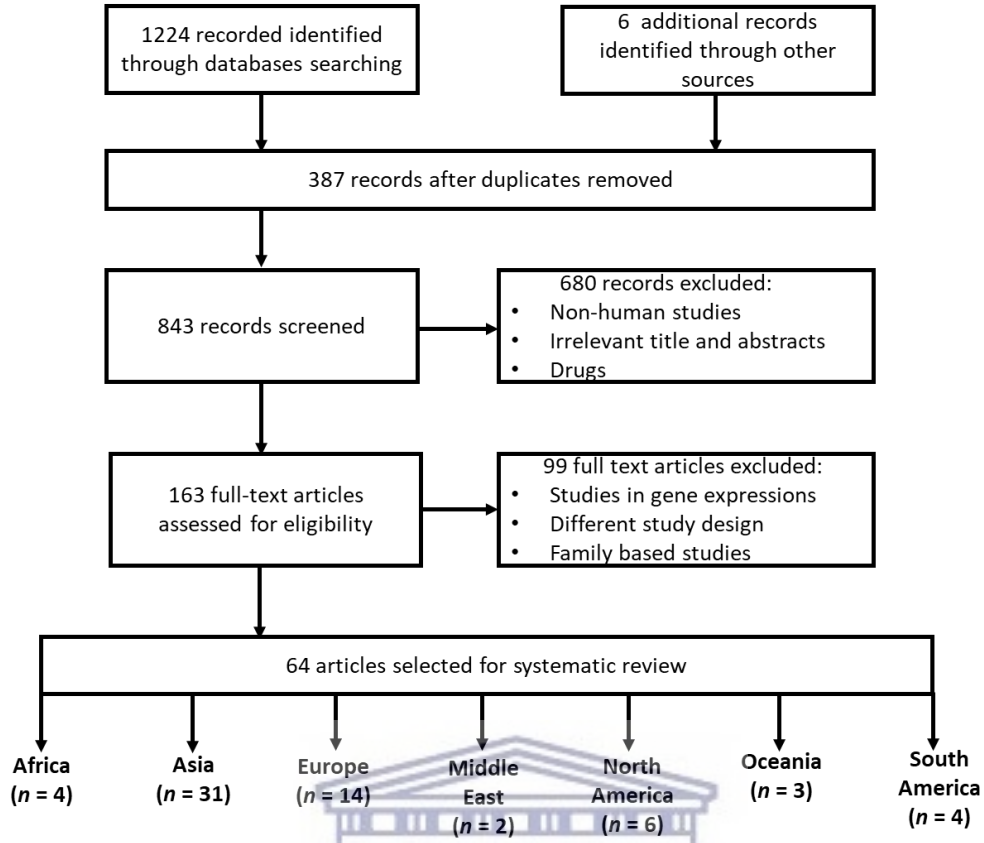

Figure 4.1. A flow diagram showing an overview of study identification, inclusion, and exclusion criteria.

Table 4.2 Main characteristics of studies included in this review.

Author, Year	Association	Country	Ethnicity	Cases	Cases with SNP	Control	Controls with SNP	P-value HWE
Africa								
Ghogomu *et al*, 2016 [34]	Yes	Cameroon	Bantu	41	38	50	5	Yes
Amrani-Midoun *et al*, 2016 [15,36]	No	Algeria	Caucasian	82	45	72	28	Yes
Nassereddine *et al*, 2015 [16]	Yes	Morocco	Caucasian	101	54	102	48	Yes
Amin *et al*, 2012 [35]	No	Egypt	Caucasian	97	40	84	37	Yes
Asia								
Arina *et al*, 2019 [37]	Yes	Indonesia	Asian	53	21	53	10	Yes
Dwivedi *et al*, 2017 [38]	Yes	India	Asian	100	29	223	39	No
Fan *et al*, 2016 [28]	Yes	China	Chinese	214	177	494	375	Yes

Wen *et al*, 2015 [39]	Yes	China	Asian	174	129	634	376	Yes
Wang *et al*, 2015 [40]	Yes	China	Asian	190	94	287	143	Yes
Cai *et al*, 2014 [41]	Yes	China	Chinese	200	161	200	139	Yes
Xi *et al*, 2013 [42]	Yes	China	Chinese	619	378	2458	1376	Yes
Zhang *et al*, 2012 [43]	No	China	Asian	189	61	165	48	Yes
Cao *et al*, 2012 [44]	Yes	China	Asian	223	158	147	98	Yes
Yin *et al*, 2012 [45]	Yes	China	Asian	670	426	682	360	No
Liu *et al*, 2011 [46]	No	China	Asian	155	97	140	66	No
Cai *et al*, 2009 [47]	Yes	China	Chinese	130	53	39	8	Yes
Lin *et al*, 2008 [48]	Yes	China	Asian	50	31	123	50	Yes
Luo *et al*, 2008 [49]	Yes	China	Asian	442	182	195	57	Yes
Tang *et al*, 2007 [50]	Yes	China	Asian	252	113	195	57	Yes
Markan *et al*, 2007 [51]	Yes	India	Asian	153	48	133	28	Yes
Hui *et al*, 2007 [52]	No	Japan	Asian	261	178	271	167	Yes
Xing *et al*, 2007 [53]	Yes	China	Asian	695	493	509	327	No
Li *et al*, 2006 [54]	No	China	Asian	26	8	30	9	Yes
Hu *et al*, 2006 [55]	No	China	Asian	110	55	115	54	Yes
Kalita *et al*, 2006 [56]	Yes	India	Asian	28	10	32	11	Yes
Lwin *et al*, 2006 [57]	No	Japan	Asian	116	77	219	155	Yes
Liu *et al*, 2005 [58]	Yes	China	Asian	100	71	100	69	Yes
Sun *et al*, 2003 [59]	Yes	China	Asian	55	49	46	32	Yes
Wang *et al*, 2002 [60]	Yes	China	Asian	105	88	46	32	Yes
Zhan *et al*, 2000 [61]	No	China	Asian	127	83	170	108	Yes
Kobashi *et al*, 2000 [62]	Yes	Japan	Asian	184	120	215	132	Yes
Gao *et al*, 1999 [63]	Yes	China	Asian	127	83	170	108	Yes
Nakata *et al*, 1998 [64]	No	Japan	Asian	173	110	184	119	Yes
Nishio *et al*, 1996 [65]	No	Japan	Asian	47	31	82	53	Yes
Europe								
Bayramoglu *et al*, 2015 [66]	Yes	Turkey	Caucasians	125	60	99	43	Yes
Husemoen *et al*, 2014 [67]	Yes	Denmark	Caucasians	4694	2463	7697	3907	Yes

Ilhan *et al*, 2008 [68]	Yes	Turkey	Turk	78	42	100	28	Yes
Marinho *et al*, 2007 [69]	Yes	Portugal	Portuguese	64	49	128	71	Yes
Nagy *et al*, 2007 [70]	Yes	Hungary	Caucasians	101	52	73	41	Yes
Demir *et al*, 2006 [71]	Yes	Turkey	Caucasians	100	67	102	59	Yes
Cesari *et al*, 2005 [72]	Yes	Italy	Caucasians	90	50	90	48	Yes
Tylicki *et al*, 2005 [73]	No	Austria/Poland	Caucasians	90	50	90	48	Yes
Yilmaz *et al*, 2004 [74]	Yes	Turkey	Caucasians	64	35	47	23	Yes
Frederiksen *et al*, 2004 [75]	Yes	Denmark	Caucasians	1267	691	7971	4120	Yes
Rodriguez-Esparragon *et al*, 2003 [76]	No	Spain	Caucasians	232	149	215	120	Yes
Kahleova *et al*, 2002 [77]	Yes	Czech Republic	Caucasians	164	82	173	87	Yes
Benes *et al*, 2001 [78]	No	Czech Republic	Caucasians	193	120	209	123	No
Zusterzeel *et al*, 2000 [79]	Yes	Netherlands	Caucasians	76	44	403	198	Yes
Middle East								
Alghasham *et al*, 2012 [80]	Yes	Saudi Arabia	Qassim	123	50	250	65	Yes
Fakhrzadeh *et al*, 2009 [81]	Yes	Iran	Asian	160	61	76	40	Yes
North America								
Perez-Razo *et al*, 2015 [82]	Yes	Mexico	Mexican	569	423	590	465	Yes
Vazquez-Alaniz *et al*, 2014 [83]	Yes	Mexico	Mixed	194	132	194	140	Yes
Deshmukh *et al*, 2009 [84]	Yes	United States	Caucasians	42	20	118	66	Yes
Canto *et al*, 2008 [85]	Yes	Mexico	Caucasians	125	89	274	213	Yes
Rajkovic *et al*, 2000 [86]	Yes	United States	American	171	29	183	32	Yes
Powers *et al*, 1999 [87]	Yes	United States	American	122	76	114	60	Yes
Oceania								
Fowdar *et al*, 2012 [88]	No	Australia	Caucasians	377	207	393	218	Yes
Ng *et al*, 2009 [89]	Yes	Australia	Caucasians	38	24	80	40	Yes
Heux *et al*, 2004 [90]	Yes	New Zealand	Caucasians	247	160	249	144	Yes
South America								
Rios *et al*, 2017 [91]	Yes	Brazil	American	96	83	85	65	Yes
Fridman *et al*, 2013 [92]	Yes	Argentina	Caucasians	75	46	150	79	Yes

Fridman *et al*, 2008 [93]	No	Argentina	Caucasians	40	25	86	47	Yes
Soares *et al*, 2008 [94]	Yes	Brazil	American	30	17	16	7	Yes

4.3.2. Association of *MTHFR* (rs1801133) and HTN reported in African Continent

In this section, we briefly summarize the evidence on *MTHFR* (rs1801133) associations based on the four available studies reporting on African populations (Table 4.2).

The first study was conducted by Amin *et al*. [35], and it was aimed at evaluating the presence of *MTHFR* (rs1801133) polymorphism and its association with HTN and myocardial infarction among participants of Egyptian origin (n = 181, <45 and ≥45 years). The study showed that there was no association between *MTHFR* (rs1801133) and HTN. The study further demonstrated that individuals with HTN were smokers and presented with impaired lipid profiles such as significantly raised levels of total cholesterol (TC), triglycerides, low-density lipoprotein-cholesterol (LDL-c), and low high-density lipoprotein cholesterol (HDL-c), in comparison to the control group. The gender of the participants was not reported in this study. The authors clearly stated the guidelines (SBP/DBP ≥ 140/≥ 90 mm Hg) that were used to define HTN. However, the method used to adjust for patients who were already on treatment was not mentioned.

The second study by Nassereddine *et al*. [16] was carried out to evaluate the association between *MTHFR* (rs1801133) variant and HTN in a Moroccan population (n = 203, range 40–87 years). The authors demonstrated a significant association between rs1801133 and HTN. It was further demonstrated that the distribution of demographic and clinical characteristics of patients did not show a significant trend in relation to HTN. Thus, the study did not adjust for confounding factors. The study reported more females (n = 77) than males (n = 24). Lastly, the study defined HTN as SBP/DBP ≥ 140/≥ 90 mm Hg. However, the authors did not provide any information about the treatment status of the cohort.

The third study by Amrani-Midoun *et al*. [15] reported a lack of association between *MTHFR* (rs1801133) and HTN in an Algerian population (n = 154, ≥42 years); however, the authors did acknowledge the impact of the small sample size used. Despite the small sample used, this study showed that there were significant differences between participants with HTN and controls with respect to age, SBP, DBP, and family history of HTN. The study was composed of more females (n = 84) than males (n = 70), and defined HTN as SBP/DBP ≥ 140/≥ 90 mm Hg. However, the method that was used for adjusting for the use of antihypertensive medication was not mentioned. Also, the genotyping method used in this study (PCR-RFLP) could be a potential limitation.

The fourth study was conducted by Ghogomu *et al*. [34], and it reported an association between *MTHFR* (rs1801133) and HTN in the native Bantu ethnic group of the Southwest region of Cameroon (n = 91, range

40–70 years). Of note, this was the only study that sampled participants from an indigenous African population. Lipid profile dispersion for all subjects reported that serum lipid levels were higher in hypertensive patients than in healthy controls. The study further demonstrated that the *MTHFR* (rs1801133) variant may influence individual susceptibility to HTN through a mechanism that involves an increase in the level of serum LDL-c. However, the sample size was very small and was likely accompanied by biasedness. Furthermore, the study did not report on the number of females/males that were sampled. HTN was defined as having elevated SBP ≥ 140 mm Hg and DBP of at least ≥ 90 mm Hg. Patients who were already placed on hypertensive medication were also categorized as hypertensive.

4.4. Discussion

The Methylenetetrahydrofolate reductase gene has been among the most studied genes associated with the development and progression of HTN [26,36]. Indeed, numerous genetic studies have investigated the association between the genetic variant of *MTHFR* (rs1801133) and the risk of developing HTN [36–38]. However, these studies reported conflicting results. In our previous systematic review, the *MTHFR* gene (rs1801133) was reported as one of the most studied genes associated with HTN among African populations [95]. Thus, in the present review, we gathered and analyzed the available genetic evidence on the association between *MTHFR* (rs1801133) and HTN among Africans and further compared the evidence with global data.

We reviewed 60 published articles that examined the association between *MTHFR* (rs1801133) and HTN. Out of 60 published articles, 47 reported a positive association between HTN and the *MTHFR* variant. However, only 4 studies were conducted in the African continent, of which 2 reported a positive association between rs1801133 and HTN [16,34]. The inconsistencies observed between these studies may be due to: (a) the limited number of relevant African studies and their relatively small sample sizes, which makes comparisons with other studies challenging. Given the small sample size in these studies, many true associations with small effects will not be significant and many suggestive associations may be false. In addition, the use of various cohorts, to maximize sample size and increase statistical power, could interfere with the biased results as some associations may be due to heterogeneity [96]; (b) the low frequency of the *MTHFR* (rs1801133) T allele observed among the African populations [97], which may be influenced by folate deficiency due to malnutrition and impaired intestinal absorption of folic acid, which are common in Africa [98]. Lastly, a study by Amrani-Midoun *et al*. [15] also suggested that these differences may be due to the epigenetic mechanisms which are involved in the gene expression predisposed by environmental factors such as lifestyle and diet. All the afore-mentioned factors may lead to failure to replicate the association of *MTHFR* (rs1801133) with disease phenotypes.

Although all included African studies [15,16,34,35] defined HTN as SBP/DBP ≥ 140/≥ 90 mm Hg, there were great differences in these studies, partly because of the criteria used in selecting participants and methods applied in each study. A study by Ghogomu *et al*. [34] and Nassereddine *et al*. [16] reported an association between *MTHFR* (rs1801133) polymorphism and HTN. However, these studies did not adjust for confounding factors such as gender, age, and smoking status. This may introduce bias, thus making it difficult to compare the findings with other studies. Furthermore, the age inconsistencies among the four African studies [15,16,34,35] may impose challenges when comparisons are made with other studies. For instance, a study by Nassereddine *et al*. [16], included 101 outpatients with a mean age of 61.6 ± 9 (range 40–87 years) and 102 age and sex-matched unrelated healthy control subjects with a mean age of 59.24 ± 10.7 (range 40–87 years); whereas a study that was conducted by Amin *et al*. [35] sampled young adults aged < 45 years and older adults aged ≥ 45 years. The use of antihypertensive medication was reported by African studies [15,16,34,35]; however, the methods used for adjustments were not mentioned. This may introduce bias when making comparisons across studies, as studies that make adjustments would not be comparable to studies that did not make adjustments. Ghogomu *et al*. [34] was the only study that was composed of participants from an indigenous African population [15,16,35], thus limiting comparisons across different racial groups, since the genetics of HTN vary across different populations and geographical regions [17,99].

Nonetheless, a recent systematic review and meta-analysis comprising of 57 studies with 14,378 patients and 25,795 control subjects examined the association between *MTHFR* (rs1801133) and HTN and revealed that the major reason for equivocal results might be the racial differences observed across the different studies [36]. In comparison with that study, the present review had the following advantages: First, there were 64 eligible studies with 16,186 hypertensive cases and 29,070 controls, which could provide more reliable conclusions. Second, since none of the previous systematic reviews and meta-analyses [26,27,100–102] focused on the indigenous African populations, we assessed the comparison among studies reported on other cohorts with the ones reported on African populations. Therefore, future studies should pay more attention to the differences in the genetic background of indigenous African populations. For this reason, our review updates information from the previous systematic reviews [26,27,100–102] with additional supplements and adjustments, which makes it a comprehensive study regarding the association between *MTHFR* (rs1801133) and HTN.

Strengths and Limitations of This Study

It must be pointed out that this is the first review that specifically assessed the effect of *MTHFR* (rs1801133) on HTN in Africa, as well as performing comparisons between African studies and available global data, which opens the door for future research. However, it should be noted that there were certain limitations to

the present analysis, which inevitably prevented more in-depth analyses. First, the sample size of some of the selected studies was relatively small. Second, variations between population characteristics, phenotypic measures, and genotypic analyses could cause bias when comparing the current findings with previous reports. Third, literature was surveyed globally; unfortunately, in Africa, we were able to identify only four studies, which suggests that there is a lack of information regarding the black African ethnic groups in relation to genetic association studies. Furthermore, out of those identified studies (n = 4), only one study by Ghogomu *et al*. [34] was composed of a purely black African population and the remaining three studies were composed of other ethnicities [15,16,35]. Thus, our meta-analysis only included a few numbers of participants who were of African origin. As such, the analysis was unlikely to produce valid results (Figure 4.2), thus we conducted a narrative synthesis of the results. This indicates that there is an urgent need to carefully plan African-specific studies with large sample sizes in order to be able to draw conclusions on the association between *MTHFR* (rs1801133) and HTN.

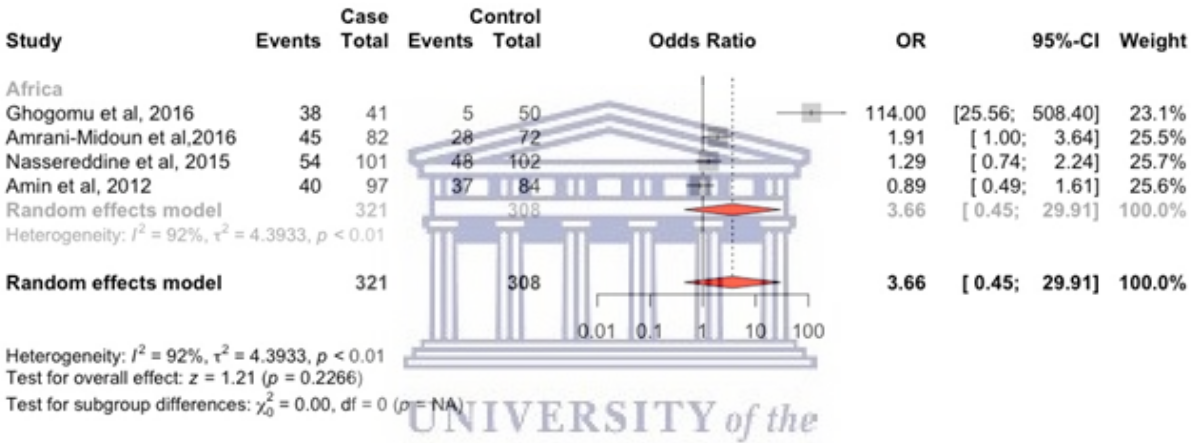

Figure 4.2. Forest plot of the evaluation for the association between the *MTHFR* (rs1801133) and HTN in the dominant genetic model (Africa). We evaluated the risk of the TT or CT genotype on HTN compared with the CC genotypes. Then, pooled Odds ratios (OR) with 95% confidence intervals (CI) and z score were performed to estimate associations. All analyses were performed using R software (Version 3.3.3, using R package meta) [103].

4.5. Conclusion

Although the association between rs1801133 and HTN was predominantly reported in other global regions, the result from the current review opened avenues to further explore a possible association between rs1801133 and HTN among individuals of African origin. Furthermore, this study has demonstrated the need to generate African-specific genomic data. Such data could provide insights into human evolution and the role of genetic variants in disease phenotypes. These data could also increase our understanding of African population genomics and highlight its potential impact on biomedical research and genetic susceptibility to disease. Thus, future studies should sample a fair number of participants that completely

represent the African population. Since African populations are well known to have high genetic diversity, because of their deep evolutionary history, and genetic differences, it is of utmost importance for future association studies to pay more attention to African genetic studies and to understand the functional and biological relevance of associated rs1801133. Moreover, improved methods need to be developed to understand and compare heritability across populations and study participants from different parts of the African continent. It is also imperative for all studies to report more detail in the protocols used to enable better replication and minimize bias between studies.

4.6. Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Publication Search, Table S2: PRISMA abstract check list, Table S3: PRISMA manuscript check list.

4.7. Author Contributions: S.E.M., B.M., and R.J. conceptualised and designed the study. S.E.M., C.M., K.Z., and B.M acquired the data. S.E.M., B.M., and S.S., analyzed and interpreted the data and drafted the manuscript. B.M, S.E.M., S.S., T.A., L.M., K.Z., J.R.S., M.B., S.N., and R.J. were involved in the critical revision of the manuscript for intellectual content. All authors have read and approved the final version of the manuscript.

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4.11. Informed Consent Statement: Not applicable

4.12. Data Availability Statement: Not applicable

4.13. Conflicts of Interest: The authors declare there are no conflicts of interest.

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CHAPTER FIVE

Original Research Manuscript

The burden of hypertension is not fully understood yet and now is a serious public health concern, especially in developing countries such as rural parts of South Africa. The establishment of clinical and context-specific interventions is needed to improve patient care to manage the disease. Therefore, the objective of this chapter was to examine the prevalence, treatment, determinants, and associated comorbidities of hypertension among the rural population of the Eastern Cape, South Africa. This manuscript was designed to address objective one.

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My contribution:

Data curation

Analysed data and interpretation

Original draft preparation

CHAPTER 5: Prevalence of hypertension and its associated risk factors in a rural black population of Mthatha town, South Africa

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Abstract

Background

The occurrence of hypertension (HTN) has been alarmingly increasing in both low and middle-income countries. Despite acknowledging HTN as the most common life-threatening risk factor for cardiovascular disease (CVD), a dearth of data is available on the prevalence, awareness, and determinants of HTN in rural parts of South Africa. The principal aim of the current study is to determine the prevalence and associated risk factors of HTN among a black rural African population from the Mthatha town of Eastern Cape Province.

Methods

This was a cross-sectional study, and individuals over 18 years of age were randomly screened using a World Health Organisation stepwise questionnaire. Sociodemographic information, anthropometric measurements, fasting blood glucose levels, and three independent blood pressure (BP) readings were measured. Blood pressure measurements were classified according to the American Heart Association guidelines. Univariate and multivariate analyses were performed to determine the significant predictors of HTN.

Results

Of the total participants (n = 556), 71% of individuals had BP scores in the hypertensive range. In univariate analysis, age, westernized diet, education, income, and diabetic status, as well as overweight/obese status were positively associated with the prevalence of HTN. However, in a multivariate logistic regression analysis only, age, body mass index (BMI), diabetic status, and westernized diet were significantly associated with a higher risk of developing HTN. Gender, age, and BMI were potential factors having a significant association with the treatment of HTN. Individuals who did not consider the importance of medicine had higher chances of having their HTN being untreated.

Conclusion

Prevalence of HTN was high among the black rural African population of Mthatha town. Gender, age, westernized diet, education level, income status, diabetic as well as overweight status were the most significant predictors of HTN.

5.1 Introduction

Hypertension drives the global burden of cardiovascular disease and is a leading cause of cardiovascular-related mortality worldwide, with 1.39 billion affected adults and 10.4 million deaths globally[1-3]. The prevalence of HTN has escalated globally with an estimated projection of a 30% increase in occurrence by the year 2025 [4]. Until recently, HTN was mainly acknowledged as a serious medical condition associated with mainly affluent regions of the world, however, this public health condition has increased drastically with three out of four individuals residing in low and middle-income countries(LMICs) being hypertensive [5]. In Sub-Saharan Africa, HTN has emerged as a major public health problem contributing to the rising number of premature deaths in this region. In particular, South Africa has been shown to have the heaviest burden of HTN with an estimated prevalence between 27-58% [1, 6]. Gutwatudde *et al*. [7] and later Gomez Olive *et al* [6] reported that the prevalence of HTN in South Africa will rise substantially if effective intervention strategies are not implemented timeously [6, 8]. These interventions include three key elements: identifying and addressing modifiable risk factors, diagnosis and screening of HTN, and finally treatment with follow-up of diagnosed participants with HTN. However, these interventions are suboptimally controlled in the rural parts of South Africa.

Indeed, if left untreated, HTN can cause stroke, dementia, renal failure, blindness, myocardial infarction to name a few [3, 9]. Despite being recognized as a major risk factor for cardiovascular disease, there is still a paucity of data available on the prevalence, awareness, risk factors, and control of HTN among rural communities of South Africa. Information about trends and potential determinants of HTN is essential for the improvement of community-based preventive strategies and management of HTN.

Current evidence indicates that HTN is a multifactorial condition influenced by many risk factors including genetic, sociodemographic, and behavioral factors [4, 10-12]. Genetic and demographic factors including age, ethnicity, gender cannot be modified while behavioral factors such as physical inactivity, unhealthy dietary choices are often modifiable [13]. Socioeconomic status (SES), a sociological construct, refers to an individual's relative position in the social hierarchy. Various indicators such as occupational group, educational attainment, level of income and wealth, and place of residence are utilised to measure socioeconomic status. Educational and socioeconomic status at the individual and parental level is reported to have an association with high blood pressure and awareness of HTN [14]. Additionally, SES has been reported to predict health behavior and access to preventive health measures [15-17]. Various studies from African countries have shown an association between SES and HTN but results are quite variable, with both positive and negative associations being reported [9, 18, 19]. Some studies have also investigated the influence of body mass index (BMI), physical activity, and diet on blood pressure patterns. Body mass index has been directly associated with a higher risk of developing HTN while physical activity is inversely associated with HTN in developed economies however findings were not consistent in low to middle-

income countries[20-23]. Furthermore, high BMI (overweight/obesity) is often associated with insulin resistance and has important implications for the development of Type 2 Diabetes Mellitus (T2DM) [24, 25] that is a growing problem across Africa affecting 19 million adults including 14.5 million in sub-Saharan Africa [26]. Both obesity and T2DM are also linked to higher mortality risks due to cardiovascular diseases [27, 28].

These biological and behavioral factors are demonstrated to have an uneven distribution across socioeconomic strata which makes them possible mediators of the observed association between sociodemographic variables and blood pressure [29]. These sociodemographic and clinical factors not only determine the prevalence of HTN but also have a drastic impact on the control of HTN. This was first observed by Adeniyi *et al* [10] who reported poor control of HTN in individuals living with T2DM in the Mthatha town of South Africa. Despite the availability of advanced diagnostic options and several therapeutic drugs, various studies have reported suboptimal BP control in rural and urban settings of SA [3, 30-33]. A large proportion of hypertensive patients remain unaware of their condition and do not receive any treatment. The primary health care system of South Africa is overburdened with ongoing infectious disease challenges along with the rising demands from increasing HTN and related complications with limited resources available [34]. As such management and control of HTN are critical in countries like South Africa, a country with extreme levels of poverty and socioeconomic inequality because of its apartheid history [35]. Information on the prevalence and factors associated with HTN among various communities is urgently needed for the development of community specific HTN preventative strategies.

The scarcity of data on sociodemographic, bio-behavioral, and comorbidities associated with HTN in rural areas of South Africa raises the need for research among rural populations for developing effective and population-based interventions for the prevention of HTN. The objective of this study, was, therefore, to determine the prevalence, treatment, determinants, and associated comorbidities of HTN among the rural population of the Eastern Cape, South Africa. Such epidemiology data is urgently required by health policymakers to tailor specific strategies for the control of HTN.

5.2 Material and Methods

5.2.1 Study Design

The present cross-sectional study included the Xhosa speaking African population from four districts (OR Tambo, Alfred Nzo, Chris Hani, Joe Gqabi) with five sub-districts and fourteen community health care centers in the Eastern Cape province, South Africa. This province is the second largest province in the country and serves a population of 7,130,480.

5.2.2 Eligibility Criteria

To be eligible for this study, patients needed to be over 18 years of age and belong to African ethnicity. Exclusion criteria were pregnant women and reluctance to participate in the study.

5.2.3 Ethical approval

The study conforms to the ethical guidelines of the Declaration of Helsinki [36] and obtained approval from ethics committees of Water Sisulu University (073/15) and South African Medical Research Council (EC028-8/2020). The participants were provided an information sheet written in both English and IsiXhosa languages with details of the purpose, the process of research, rights of the participants, and details of the contact person for any inquiry before granting a written informed consent.

5.2.4 Sample Size and sampling

The appropriate sample size was estimated using the following formula:

$$\boldsymbol{n = \frac{p(1-p)z^2}{d^2} = \frac{0.30(1-0.30)1.96^2}{0.05^2} = 322}$$

Where z is the confidence level, p is the expected proportion of patients with HTN, and d is the margin of error. p was set at 0.30 and the desired precision is 5%. The calculation was done at a 95% confidence level. A total of 556 participants were included just to compensate for incomplete records.

5.2.5 Sampling procedure

A total of 556 participants, were randomly selected in series at the hospital outpatient department (without having prior knowledge of their HTN status). However, 19 participants were not included in the further analysis due to incomplete data.

5.2.6 Data collection

A face-to-face interview was conducted, and informed consent was obtained from all the participants and after consenting, all respondents were medically examined by trained survey staff**.** Consent forms and the World Health Organisation (WHO) STEPwise questionnaire were uploaded onto the Research Electronic Data Capture (REDCap) a web-based application for building and managing online surveys and databases [37]. The questionnaire included information on gender, age, race, marital status, level of education, monthly income, the status of employment, and behavioral characteristics (alcohol consumption, physical activity, dietary intake, knowledge, and beliefs of HTN, and its treatment). Additionally, information about anthropometric measurements (weight and height), was also included. A pilot study was performed on 180 participants (not included in the study) to ascertain the validity of the instrument.

5.2.7 Assessment of Overweight/Obesity

Participants were weighed bare feet with light clothing to the nearest 0.1kg using a standard beam balance. Height was measured to the nearest 0.1cm on the mounted Stadiometer, and then BMI was calculated. Participants were categorized into normal, overweight, and obese according to the WHO standards[38]. Participants were considered as overweight if BMI was 25-29.9 kg/m^2 and were categorized as obese if their BMI was $\geq 30.0 kg/m^2$. Participants with BMI ≤ 25 were either classified as normal or underweight.

5.2.8 Diagnosis of type 2 Diabetes Mellitus (T2D)

A blood sample was taken after an overnight fast. The diagnosis of T2D was confirmed according to the criteria of the American Diabetic Association, 2011[39]. Participants were categorized as normal (fasting plasma glucose level (FPG) < 5.6 mmol/L), prediabetic (FPG level between 5.6 - to 6.9 mmol/L), and diabetic (FPG level >7 mmol/L).

5.2.9 Measurement and definition of Blood Pressure

Blood pressure was measured using an automated blood pressure monitor (ROSSMAXCF155/709 model) according to the standard operating procedures. Before the measurement, patients were asked to rest for five minutes with the arm at the level of the heart and the feet together. Screening and diagnosis of HTN were defined as per guidelines provided by the American Heart Association (AHA), 2018 [40]. A mean of three repeated BP measurements was used in all calculations and analyses. Thereafter, participants were categorized into three categories based on their BP: 1) elevated blood pressure (systolic blood pressure (SBP):120-129 mm Hg and diastolic blood pressure (DBP)<80mm Hg), 2) HTN grade 1 (SBP:130-139 mm Hg and DBP: 80-89 mm Hg), and 3) HTN grade 2 ≥ 140 mm Hg and ≥ 90 mm Hg. Each subject was asked questions on the awareness and treatment of HTN. Clinical records of participants identified as hypertensive were assessed for three months post initial diagnosis to confirm the disease state.

5.2.10 Socioeconomic and environmental variables

Information about sociodemographic and environmental variables was collected during personal face to face interviews using a WHO stepwise questionnaire uploaded on REDCap. Age (years) was considered as a continuous variable race was self-reported by participants as per historical group categorisation in South Africa.

Four self-reported sociodemographic factors were assessed: level of education, employment, income, and marital status. Education variables were recorded as one of four categories from no schooling to primary school (category1), high school (category 3), and tertiary (category 4). Individuals were categorized as employed or unemployed based on their employment status Participants were defined as unemployed if they did not have any occupation in either the formal or informal sectors. The income group was categorized

into <R1000 ($65) and ≥R1000 ($65) according to the total monthly amount earned/accruing to an individual's household. Marital status was recorded as single or in a relationship.

Self-reported levels of physical activity were measured and categorized into ≥150 minutes per week or <150 minutes based on the WHO recommendations [41]. The dietary intake of participants was obtained through the 24-hour recall method and the information was used to describe adherence to the westernized diet. Westernized diet is defined as a diet rich in saturated fats, refined grains, sugar, and salt with reduced consumption of fruits and vegetables [42]. Diet was further categorized into three categories:1) low intake of westernized diet referred to as a diet low in salt, refined grains and high in veg/fruit or with only one of the risk factors;2) moderate intake of a westernized diet with two of the risk factors; and 3) high intake of westernized diet, with all three of dietary risks present.

5.2.12 Data Analysis

Statistical package for social sciences (SPSS) windows version 16.0 (SPSS, Inc., Chicago, IL, USA) was used to analyze the data. The unadjusted odds ratio was utilised to calculate the association between HTN and socio-economic demographic factors. Multivariate regression analysis was carried out between dependant (HTN) and independent variables (socio-economic and demographic). Significance was tested at 95%, $p<0.05$ was taken as significant.

5.3 Results

5.3.1 Participant's sociodemographic and clinical characteristics and levels of blood pressure

The study sample included 556 black African individuals over 18 years of age, and women comprised the bulk of the sample (85.1%). Table 5.1 provides information about the sociodemographic and clinical characters of the participants along with the status of their blood pressure. Three-quarters of the participants (71.0%) had blood pressure readings in the hypertensive range (Table 5.1). More than one-third of participants had BP readings that met the criteria for HTN grade II and almost half of them belong to the elderly age group (>50 years of age; Figure 5.1a and Figure 5.1b). However, only 44.3% of the total participants with BP in the hypertensive range were aware of their hypertensive status.

Overall, participants were predominantly single (61.1%), unemployed (56.1%), and with limited monthly income (58.9% earned<R1000). In terms of education, 52.9% of participants had completed high school, with only 17.7% progressing to post-school education. In terms of clinical characteristics, almost three-quarters of participants were either overweight or obese and about 40% were categorized as prediabetic or diabetic. More than half of the participants were physically inactive and followed a westernized diet with a high intake of salt and refined carbohydrates and a low intake of fruit and vegetables. Furthermore, almost 82% of individuals with increased consumption of a westernized diet were hypertensive. Around 84.5% of the hypertensive participants considered that HTN cannot be controlled with medication, though most of

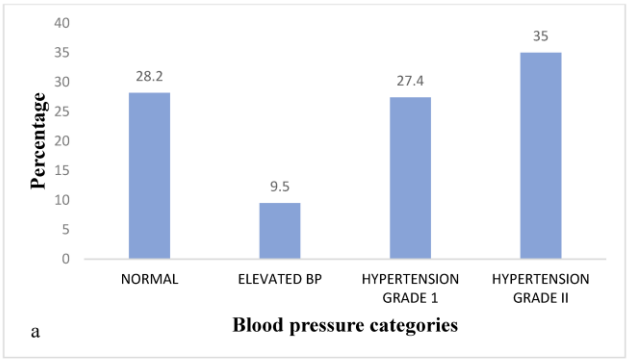
them (70.6%) were hypertensive. Only 22.3% of the participants considered that HTN can be controlled with diet.

Table 5.1. Sociodemographic and clinical characteristics of the population along with the status of their blood pressure.

Variables	Total sample	Blood pressure scores		
	%	Normal BP	Hypertension	p-value
Gender				
Male	14.9%	34.9%	65.1%	0.061
Female	85.1%	25.1%	74.9 %	
Age categories				
18-35	26.0%	37.5%	62.5%	
36-49	31.4%	33.7%	66.2%	<0.001
50-64	30.2%	20.0%	80.0%	
≥ 65	12.6%	14.9%	85.0%	
Education				
None/primary	29.4%	23.9%	76.1%	0.215
High school	52.9%	26.3%	73.7%	
Tertiary	17.7%	33.7%	66.3%	
Relationship status				0.984
Single	61.1%	26.5%	73.5%	
In a relationship	38.9%	26.6%	73.4%	
Employed				0.948
Yes	43.9%	26.4%	73.6%	
No	56.1%	26.7%	73.3%	
Monthly income				
<R1000	58.9%	22.7%	77.3%	0.039
≥ R1000	41.1%	30.4%	69.6%	
Body Mass Index				

Normal	27.2%	42.9%	57.1%	<0.001
Overweight	25.2%	32.3%	67.1%	
Obese	47.6%	17.1%	82.9%	
FPG levels				
Normal range	59.3%	30.8%	69.2%	0.037
Prediabetic range	21.8%	18.8%	81.2%	
Diabetic range	18.8%	24.8%	75.2%	
Physically active				
No (<150 minutes)	53.5%	25.3	74.7%	0.361
Yes (≥ 150 minutes)	46.5%	28.7%	71.3%	
Adherence to Westernized diet				
Low	11.9%	52.9%	47.1%	<0.001
Moderate	35.1%	28.9%	71.1%	
High	53.1%	18.1%	81.9%	
Beliefs about hypertension control				
Hypertension cannot be controlled with medication	84.5%	29.3%	70.6%	0.342
Hypertension can be controlled with diet	22.3%	26.2%	73.7%	0.969

[1] Chi-square tests of association were conducted; significance is determined at $p<0.05$ (overall, for a given parameter)



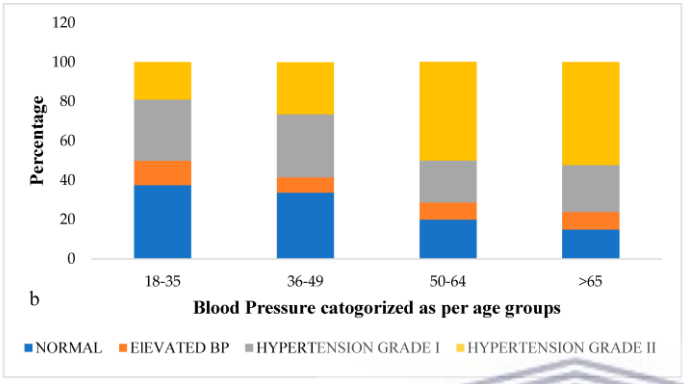


Figure 5.1. Distribution of participants according to their Blood pressure.

Table 5.1 shows significant associations of age ($p < 0.001$) westernized diet, as well as income (0.039), diabetic and overweight/obese status with the prevalence of BP scores in the hypertensive range. However, other variables such as education ($p = 0.215$), employment status ($p = 0.948$), physical activity ($p = 0.361$), HTN beliefs among participants such as the importance of medicine ($p = 0.342$), and dietary control ($p = 0.969$) did not show any significant association with the BP scores in the hypertensive range. However, gender demonstrated a marginally significant association ($p = 0.061$) with BP scores.

Variables associated with hypertensive status at $p < 0.1$ were entered into a multivariate logistic regression model (Table 5.2). In this model, the only variables that remained significantly associated with hypertensive status were age, BMI, diabetic status, and the westernized diet. Specifically, participants between 50-64 years of age [p=0.039, aOR 1.87 (1.03-3.39)], and ≥ 65 years of age [(p=0.009, aOR 3.20 (1.34-7.63)] had almost doubled and more than tripled the odds of being hypertensive, respectively relative to the participants of 35 years of age or younger. Individuals who were obese also had more than tripled the odds of being hypertensive relative to those with a normal BMI (p = <0.001, aOR = 3.52; (2.01-6.18)]. Subjects with a blood glucose reading indicative of diabetes had more than doubled [(p = 0.034, aOR = 2.24; 1.06-4.72)] the odds of being hypertensive as compared to those with normal blood glucose readings. Finally, participants who were moderately or highly adherent to a westernized diet with a high intake of salt, refined carbohydrates, and low intake of fruit and vegetables also had significantly greater odds [(p = <0.001,

OR=5.35, 2.85-10.05)] of being hypertensive. Additionally, high income (≥R1000) also indicated to increase the likelihood of HTN though with the borderline significance [(p=0.087, OR=1.47; 0.95-4.29).

Table 5.2. Multivariable logistic regression model of sociodemographic and clinical factors associated with hypertension.

Variable	**aOR[1]**	**95% CI**	**p**
Gender: Reference: female	1		
male	1.18	0.63-2.21	0.609
Age categories: Reference 18-35	1		
36-49	0.80	0.46-1.40	0.439
50-64	1.87	1.03-3.39	0.039
≥ 65	3.20	1.34-7.63	0.009
Income: Reference < 1000			
≥1000	1.47	0.95-4.29	0.087
BMI: Reference normal			
Normal	1		
overweight	1.53	0.85-2.73	0.149
Obese	3.52	2.01-6.18	<0.001
Blood glucose: Reference Normal			
Normal range	1		
Prediabetic range	1.04	0.58-1.85	0.907
Diabetic range	2.24	1.06-4.72	0.034
Westernized diet: Reference Low			
Low	1		
Moderate	2.94	1.57-5.51	0.001
High	5.35	2.85-10.05	<0.001

1 aOR = adjusted odds ratio

5.3.2 Potential variables affecting treatment status of hypertension

Among hypertensive participants, we then explored potential factors that were associated with untreated HTN (Table 5.3).

The given variables such as gender (p=0.002), age (<0.001), and education (p=0.052) (Table 5.3) illustrated a significant association with treatment status among hypertensive participants. However, relationship status, unemployment, income level, diabetic status, physical activity, and westernized diet pattern of the participants did not have any significant association with the treatment status. Interestingly, although knowing the importance of medication did not have any significant association with BP scores in the HTN range, however, this variable certainly had been observed to have a significant association with the treatment of HTN. In Table 5.3 multiple logistic regression analysis has also been utilised to investigate the significant predictors of HTN being untreated.

It is indicated that being male increases the risk of being untreated [(p=0.034, OR 2.93 (1.90-7.95)]. However, participants who belong to the age group 50-64 [(p=0.035, OR 0.45 (0.22-0.95)] and the age group ≥ 65 [(p=0.000, OR 0.190 (.07-0.48)] had lower risks of being untreated. It has been observed from the odds ratio that being overweight [p=0.109, OR 0.51 (0.23-1.16)] and obese [(p=0.002, OR 0.30 (0.15-0.64)] lowers the odds of being untreated. Similarly, those who did not understand the value of medication and its role in HTN management had higher odds of being untreated [(p=0.026, OR 3.02 (1.5-6.1)].

Table 5.3. Sociodemographic and clinical characteristics associated with untreated hypertension among hypertensive participants.

Variables	**Bivariate associations[1]**			**Multivariate associations[2]**		
	Treated hypertension (43.6%)	**Untreated hypertension (56.4%)**	**p-value**	**aOR**	**95% CI**	**p**
Gender						
Female	46.9%	53.1 %	0.002	1		
Male	19.4%	80.6%		2.93	1.90-7.95	0.034
Age categories:			<0.001	1		
18-35	25.0%	75.0%				
36-49	34.0%	66.0%		0.85	0.40-1.80	.666
50-64	52.6%	47.4%		0.45	0.22-0.95	.035
≥ 65	66.7%	33.37%		0.19	0.07-0.48	.000
Education			0.052			
None/primary	52.4%	47.6%		1		
High school	37.8%	62.2%		1.57	0.86-2.67	0.140
Tertiary	46.7%	53.3%		1.64	0.68-3.92	0.268
Relationship status			0.454			
Single	45.3%	54.7%		-		
In a relationship	41.1%	58.9%				
Employed			0.127			

yes	38.7%	61.3%				
no	47.2%	52.8%				
Monthly income			0.474			
< R1000	42.1%	57.9%				
≥ R1000	46.1%	53.9%				
Body Mass Index			<0.001			
Underweight/normal	25.8%	74.2%		1		
Overweight	37.2%	62.8%		0.512	0.23-1.16	0.109
Obese	52.0%	48.0%		0.304	0.15-0.64	0.002
FPG levels			0.163			
Normal range	40.2%	59.8%		-		
Prediabetic range	42.9%	57.1%				
Diabetic range	54.1%	45.9%				
Physically active			0.419			
No (<150 minutes)	45.7	54.3%		-		
Yes (≥ 150 minutes)	41.3%	58.7%				
Adherence to westernized diet			0.106			
Low	63.0%	37.0%		-		
Moderate	42.2%	57.8%				
High	41.7%	58.3%				
Beliefs about hypertension control						
Hypertension cannot be controlled with medication	57.4%	42.6%	0.026	3.02	1.50-6.09	0.002
Hypertension can be controlled with diet	40.0%	60.0%	0.489	-	-	-

[1] Chi-square tests of association were conducted

[2] Multivariable logistic regression, with variables associated with untreated hypertension at p<0.1 entered into the model

5.5 Discussion

Hypertension is a predominant marker of complex vascular diseases and is a serious public health threat in South Africa [8, 43]. Additionally, it is an independent and preventable risk factor for all causes of premature deaths [44]. The burden of HTN is further exacerbated by the limited information available on the prevalence, treatment, and potential determinants of HTN in rural communities. Such epidemiological data on of the prevalence and factors associated with HTN are urgently required for the development of community-based interventions. This study, therefore, aimed to evaluate the prevalence and the biological and sociodemographic variables of HTN among the residents of Mthatha, South Africa.

The current study not only reports on the associated factors of prevalence but also assesses the potential predictors determining the treatment status of HTN. The high prevalence of HTN (71%) among residents of rural communities is consistent with the previously reported prevalence in the South African Demographic and Health Survey for non-urban black South Africans [35, 45]. However, previous studies from rural areas of Sub-Saharan Africa have reported lower rates of HTN prevalence that ranges from 5%-52% [46-48]. Nonetheless, the prevalence of HTN in South Africa is reported to be 30.4% as per the recent South African National Health and Nutrition Examination Survey (SANHANES) [49-51]. As such, results presented in the current study confirmed previous findings and also highlight a serious concern of the rising prevalence of HTN in rural South Africa [49, 52-54].

Various studies have indicated that in comparison to the developed countries, developing countries are experiencing a higher rise in prevalence rates [55-57] without any further improvement in awareness and control rates. This trend is not surprising considering urbanisation, unhealthy lifestyle and dietary habits, and their consequent adverse health effects on the health of the population. Furthermore, limited health services due to inadequate funds, poor infrastructure, lack of equipment compounded with medical illiteracy are major obstacles for preventing and controlling HTN. Likewise, with South Africa being a middle-income country, results from this study supports the view that there is an epidemiological transition of non-communicable diseases to low- and middle-income countries including their rural population.

Moreover, the influence of gender on the risk of developing HTN has not been well documented with contradictory results being reported regarding the association between gender and prevalence of HTN. Most findings conducted in different districts of South Africa reported females having a lower prevalence of HTN than males [58-63]. In contrast, Alberts *et al*. and later Mkhonto *et al*. showed that the prevalence was low among males [64, 65]. The current study is consistent with these studies, reporting a higher prevalence of HTN among females than males.

Additionally, many studies have demonstrated an association between age and risk of developing HTN with HTN being more prevalent among older people [53, 58, 66]. These findings were also supported by the World Health Organisation, 2012 reporting that 75% of adults aged > 50 years of age are hypertensives in

South Africa [16]. The results of this study also confirmed that age is a significant predictor of higher HTN prevalence. Participants belonging to 50-64 years and of >65 years of age had a higher risk of having HTN in comparison to the young participants. It has been suggested that an increase in vascular resistance contributes to an increase in HTN in elderly people [67, 68]. Most chronic diseases occur during this stage of life because of the interactions between multiple disease processes and loss of physiological functions [69].

Zhou *et al* and Mashiane *et al*. reported a high prevalence of overweight and obesity in South African rural populations in all age groups [70, 71]. Similarly, our study also revealed a high prevalence of obesity in the studied rural population. Previous studies suggested that higher BMI is a major risk factor for developing HTN [44, 58, 72] and there is a linear relationship between an increase in BMI and HTN [40]. Additionally, it has also been demonstrated that obesity and visceral adiposity has been positively correlated with the renin-angiotensin system that controls blood pressure [73-75].

Furthermore, It has also been reported that there is a substantial overlap between etiology of HTN and diabetes because of their common metabolic pathways and shared risk factors [76]. In a previous study [10] from the same area, a high prevalence of HTN (81%) among individuals with type 2 diabetes has been reported. Another study reported that individuals with diabetes have almost double the chances of developing HTN than those without diabetes [77]. In addition to this, a positive association has been identified between obesity diabetes, , HTN, and cardiovascular disease [78]. Concerning the biological determinants of HTN, the current study agrees with the previous findings of associating obesity and diabetes with an increased risk of developing HTN. A high prevalence of non-communicable diseases (NCDs) such as HTN, obesity, diabetes, and other cardiovascular diseases in urban and rural parts of South Africa is a major public health concern because of the serious added economic burden with these diseases [78, 79]. Considering epidemiological and nutritional transition in sub-Saharan Africa, the World Health Organisation has also projected that Sub-Saharan African countries will experience a high prevalence of cardiovascular diseases in the next decade [80, 81].

Sociodemographic variables such as education, employment, household income, and household assets have also been reported to influence blood pressure levels [29, 82, 83]. Some studies have revealed an inverse relationship between socioeconomic status and prevalence of HTN [84] and have indicated that the risk of cardiovascular disease tends to be higher among individuals of low socioeconomic status than those belonging to a higher socioeconomic status [85, 86]. Regarding sociodemographic variables, the current study reports that relationship status, education, and employment of participants are not significant predictors of HTN. However, the income level is demonstrated to have a positive association with the prevalence of HTN. In a multivariate logistic regression analysis, participants with a higher income level (≥R1000) are more likely to be at increased risk of HTN with borderline significance reported. It could be

due to the different dietary habits of the two groups. These findings could also be explained by the fact that higher socioeconomic status does not always relate to a better nutritional status of a population but could lead to inappropriate nutritional patterns which may predispose them to the development of cardiovascular disease [87]. Furthermore, it has also been shown that higher income groups may be at higher risk of developing HTN due to the high consumption of processed foods along with their sedentary lifestyle [88]. Contradictory findings between the current study and previously reported studies could be due to the different measures of socioeconomic status (SES) in different settings. Individuals with low SES may not be able to buy expensive healthy foods or limited access to health resources [89].

The high consumption of the westernized diet is likely to have an adverse impact on the health of the South African population. These findings have also been reported in other African populations[3, 90-93]. This trend is mainly because of globalisation and increasing adherence to the westernized diet that has been reflected in the rapid expansion of fast-food restaurant chains in semi-urban and rural settings of South Africa and Sub-Saharan Africa [94]. Our study also reports that a high proportion (about 88%) of the studied population consumed moderate to high amounts of westernized diet. Further, multivariate regression analysis showed that moderate to high consumption of westernized diet was associated with an increased risk of developing HTN.

There is very limited information available about the awareness, treatment, and several other predictors of HTN treatment in South Africa. Various studies [95, 96] have emphasized the importance of a better understanding of awareness, treatment, and control for improving the management of HTN. This is critical to understand and address barriers in the treatment of HTN, therefore, in this study potential determinants of HTN treatment were also investigated. Being male was associated with higher odds of untreated HTN. This could be due to better utilisation of health facilities by women as suggested by previous studies [94, 97]. It was assessed that being male tripled the chances of being untreated than females. These findings agree with another study that demonstrates that women may be better receptive to the treatment and have regular visits to health care facilities [98]. Likewise, another study by Adeniyi *et al*. (2015) also agrees that females are more likely to visit health care facilities [94]. Traditional cultural factors in Africa could also be responsible for the underutilisation of health facilities among males; being the breadwinners, males hardly go to the health care facilities unless they are sick. [99, 100]. It is evident from various studies that education is a vital component of health and education elements should always be included in public health promotion and for the reduction of health disparities [101, 102]. Various studies have suggested that lower education level influences the treatment and control of HTN through limited knowledge of disease preventive measures, unhealthy diet, psychosocial stress due to hazardous occupations[103, 104].

In contrast, this study showed that higher education level seems to increase the odds of being untreated however, only high school education was a significant predictor when compared to primary level education.

The relationship between education and health is quite complex, it is generally assumed that an educated person will be more knowledgeable about health aspects and receptive to new drugs and treatment [105, 106]. However, these observations could vary in different races and environments; the population in the current study is poor, might not have quality education and better access to health facilities. Moreover, the association was non-significant in multivariate analysis, therefore a greater sample size is required to make any conclusion.

Additionally, it has been suggested that overweight/obese individuals are more likely to have their blood pressure documented as compared to normal-weight individuals and recoding of blood pressure measurements could be associated with the treatment. Moelnaar *et al*. and Rose *et al*. reported that obese individuals are more likely to receive HTN treatment[107, 108]. Likewise, the current study also reveals that being overweight/ obese reduces the odds of being untreated than healthy individuals.

Successful implementation of health programs relies on the knowledge, perceptions, and beliefs of any community; only a very few studies in South Africa have focused on the existing information that the community has on HTN [109-111]. Therefore, in the current study information was also taken about patients' beliefs in controlling HTN with medicine and the importance of dietary control. A small proportion of participants valued medicine for the treatment of HTN and this belief did not have any significant association with having HTN however, participants who do not give value to the use of medication for the treatment of HTN, had higher odds of having untreated HTN. Beliefs about illness and medicines are interrelated and may influence compliance. Beliefs of medicine and HTN are not only predictive of compliance but also important in achieving concordance and could be a target for achieving interventions to improve compliance [112]. Current findings in the poorly resourced area of the country reflect the poor health status of individuals living with NCDs and highlight the threat of existing vascular disease. Therefore, demand urgent action in terms of prioritizing quality health services to rural communities.

5.6 Conclusion

Our results suggest that the prevalence of HTN was high among the black rural African population of Mthatha Town. It is revealed that age, income, westernized, higher levels of blood glucose and BMI are positively associated with HTN. These findings also highlight the changing patterns of dietary habits among the rural communities of South Africa. Additionally, it has also been demonstrated that gender, age, education, BMI, and belief in controlling HTN with medication are key predictors of determining the treatment status of HTN. This study showed that about half of the participants were unaware of their HTN status and as such highlights the urgent need for HTN education, screening, and control within the Mthatha area.

5.6.1 Strengths and limitations of the study

The current study addresses the problem of HTN in the rural community of Mthatha town in Eastern Cape Province, South Africa. It highlights the critical requirement for policymakers to recognize the potential determinants of HTN and its treatment status for the management of HTN in various communities. This study had certain limitations, such as men were underrepresented that prevented us from a complete understanding of the gender-based contribution in the prevalence of HTN along with the inability to identify their specific needs. Secondly, we could not do an in-depth assessment of dietary habits and physical activity patterns, therefore, findings should be explicated with caution. Furthermore, this was a cross-sectional study, and the identification of causal associations was not possible therefore, future studies are needed to investigate the evolution of HTN and its management in the same setting.

5.7 Author Contributions:

Conceptualisation: J.R.S. and R.J.; Methodology: T.A.; J.R.S. and R.J. REDcap Software: E.N.; Formal Analysis: B.M.; J.R.S. and R.J. Data curation: S.E.M.; J.R.S. and R.J. Original draft preparation: J.R.S. and R.J. Review and Editing: C.M., E.N., B.M., M.B., R.J. All authors have read and agreed to the published version of the manuscript.

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5.9 Institutional Review Board Statement:

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Water Sisulu University (073/15; 1 February 2019) and South African Medical Research Council (EC028-8/2020; 27 October 2020).

5.10 Informed Consent Statement:

Informed consent was obtained from all subjects involved in the study. Acknowledgments: We would like to thank the staff of primary health care facilities in Mthatha Town for their support and contributions towards the successful completion of the study.

5.11 Conflicts of Interest:

The authors declare no conflict of interest.

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CHAPTER SIX

Original Research Manuscript

Recent epidemiological evidence has shown that minimal work has been done on the influence of inter-individual genetic variations on the progression and development of hypertension in developing countries. The manuscript presented in this chapter sought to investigate the role of identified *ACE* and *AGT* polymorphisms in predicting HTN in the Xhosa South African population. These genetic polymorphisms in the RAAS pathway may play a role in predicting a person's vulnerability to hypertension. This manuscript was designed to address objective five.

The chapter, as presented here, has been submitted and is under review in Genes journal (**2022**), impact factor 4.1 (genes12040543)

My contribution:

Data curation

Analysed data and interpretation

Original draft preparation

CHAPTER 6: Association analysis of *ACE* and *AGT* gene polymorphisms with hypertension in a South African population.

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Abstract

Background

Hypertension (HTN) is a main cause of cardiovascular related morbidity and mortality worldwide, with a prevalence increasing at an alarming rate in both middle- and low-income countries. Various factors, including environmental and genetic factors, have been attributed to play a significant role in the increasing prevalence of HTN. Single nucleotide polymorphisms (SNPs) within RAAS genes encoding angiotensinogen (*AGT*) and angiotensin-converting enzymes (*ACE*) are reported to have a significant association with HTN; however, there are limited studies done on South African populations. Therefore, this case-control study aimed to investigate the role of intronic SNPs of *ACE* (rs1799752 & rs4344) and *AGT* (rs2004776 & rs3789678) genes with HTN in the Xhosa population of South Africa.

Methods

These SNPs were genotyped among 90 hypertensive cases and 87 normotensive controls, using TaqMan genotyping Assays and Real-Time polymerase chain reaction (PCR).

Results

A significant association for the SNPs rs2004776 and rs3789678 but not for rs4344 with HTN in SA population was observed in a multivariate analysis.

Conclusion

There might be a possible role of the *AGT* gene polymorphisms in predicting a person's vulnerability to HTN, in the studied population. The present study represents the first report on genetic association studies on the *ACE* and *AGT* gene polymorphisms with HTN in Xhosa South African population.

6.1 Introduction

Cardiovascular diseases (CVD) are the leading cause of deaths globally, accounting for 18.6 million deaths worldwide in 2019 [1, 2]. HTN drives the global burden of cardiovascular disease and is a leading cause of cardiovascular-related mortality worldwide, with 1.39 billion affected adults and 10.4 million deaths globally [3]. HTN disproportionally affects those in low- and middle-income countries (LMICs) compared to those in high-income countries (HICs), with approximately two-thirds of hypertensive individuals living in LMICs [4, 5]. It has emerged as a major public health issue in Sub-Saharan Africa, contributing to the region's rising number of early deaths with an estimated prevalence of 27–58% in South Africa having the highest burden of HTN [6, 7]. More alarming is that HTN is referred to as a "silent killer," with 46% of adults being oblivious of their HTN status [8, 9]. Despite having an advanced understanding of the pathogenesis and the management of HTN, there is still a progressive rise in its prevalence.

Various factors including environmental and genetic factors have been attributed to play a significant role in the increasing prevalence of HTN [10-12]. Although, the role of different genetic factors in the regulation of HTN is still unknown the genes encoding the main components of the renin-angiotensin-aldosterone system (RAAS) are deemed the most potential candidate genes considering the profound role of RAAS in circulatory homeostasis [13, 14]. The RAAS hormonal system has been involved in long-term blood pressure regulation [15] and the etiology of HTN by controlling sodium and water retention, oxidative stress, fibrosis, and inflammation; and systolic blood pressure [16]. RAAS regulates blood pressure via a cascade of reactions involving the activation of various key proteins [17].

Proteins such as angiotensinogen (gene: *AGT*, protein: *AGT*), angiotensin-converting enzyme (gene: *ACE*, protein: *ACE*), renin, angiotensin I, angiotensin II, angiotensin receptor type I, and angiotensin receptor type II are significant components of RAAS. Renin cleaves *AGT* into angiotensin I. The *ACE* then cleaves angiotensin I into angiotensin II, which causes vasoconstriction and inhibits bradykinin-modulated vasodilation, resulting in elevated blood pressure. The polymorphisms in various genes within RAAS are reported to have a significant association with HTN. For example, *ACE* and *AGT* genes have been investigated in various ethnic populations, and accumulating studies report that polymorphisms in these genes may be associated with an increased risk of developing HTN [10, 18, 19]. The *ACE* (17q23.3) and *AGT* (1q42-q43) genes encode for *AGT* and *ACE* protein, respectively [20]. SNPs within the *AGT* gene, such as rs3789678, (Guanine (G)→ Adenine (A) at position 5855), and rs2004776, the (Thymine (T) → Cytosine (C) at position 6635), have been investigated for their causal role in HTN [21]. Furthermore, two polymorphisms in the *ACE* gene insertion/deletion (I/D) (rs1799752) (the presence or absence of a 278-bp Alu repetitive region) and the rs4344 (G to A/C substitution at position 231) have also been reported to be associated with HTN in various populations [22, 23].

In a recent meta-analysis conducted by Mabhida *et al*. [14] it was reported that only a few studies (eight studies on *AGT* gene and seven studies on *ACE* gene) had evaluated the involvement of different SNPs of *AGT* and *ACE* gene with the development of HTN in African populations. To date, only one study [10] reported an association of rs2004776 (*AGT* gene) SNP with HTN in an African population (Uganda). However, other studies performed on African populations exhibited no association of *AGT* gene polymorphism with the risk of developing HTN [24-27]. *ACE* polymorphisms have been widely studied in various African populations [28-30], and it has been demonstrated that rs1799752 could be a potential genetic predictor for the risk of developing HTN [31, 32] however, conflicting results have been reported in other studies [28, 33, 34]. At the same time, no studies have been reported on the association of rs3789678 and rs4344 polymorphisms with HTN in the South African population. Hence, the need to fill that gap and to assess the associations of these variants in the Xhosa population of South Africa.

Therefore, this study aims to investigate the possible role of inter-individual genetic variants of *AGT* and *ACE* genes with HTN status by utilizing TaqMan SNP genotyping assays and quantitative real-time polymerase chain reaction (q-RT-PCR) for gene expression. We hypothesize that the polymorphisms of *AGT* (rs2004776 & rs3789678) and *ACE* (rs4344 & rs1799752) will be associated with the risk of developing HTN.

6.2 Materials and Methods

6.2.1 Study design

The present case-control study included participants belonging to the rural Xhosa ethnic group from four districts (OR Tambo, Alfred Nzo, Chris Hani, Joe Gqabi), and five subdistricts, involving fourteen community health centres in Mthatha in the Eastern Cape Province of South Africa.

6.2.2 Eligibility Criteria

The study included only participants of the rural Xhosa population over 18 years of age and excluded pregnant women and those who were reluctant to participate.

6.2.3 Ethical Clearance

The study adhered to the ethical guidelines of the Declaration of Helsinki [35] and obtained approval from the South African Medical Research Council ethics committee (EC028-8/2020). Before the participants provided written informed consent, essential information about the participants' rights, research purpose, and research process was provided in two different languages (English and isiXhosa).

6.2.4 Data Collection

Data were collected via the World Health Organisation (WHO) STEPwise amended questionnaire and deposited into the Research Electronic Data Capture system (REDCap). Data collected included information on sociodemographic factors (age, gender, race) and anthropometric measurements (waist circumference, hip circumference, height, weight, systolic blood pressure, diastolic blood pressure).

6.2.5 BMI and Obesity Profiles

Anthropometric measurements such as body weight and height were taken to analyze Body Mass Index (BMI) and Waist Hip Ratio (WHR). A conventional beam balance was used to weigh participants barefoot in light clothing to the nearest 0.1 kg. On the mounted Stadiometer, height was measured to the nearest 0.1 cm, and BMI was determined by using the following formula: weight/(height)². Participants were categorized into normal, overweight, and obese according to the WHO standards [36]. Participants were considered as overweight if BMI was 25-29.9 kg/m^2 and were categorized as obese if their BMI was ≥ 30.0kg/m^2. Participants with BMI ≤ 25 were either classified as normal or underweight. The waist circumference measurement was taken approximately halfway between the lower edge of the last palpable rib and the top of the iliac crest [37]. The measurement of the hip circumference was obtained around the broadest part of the buttocks. The waist-hip ratio was calculated using the following formula: waist circumference/hip circumference.

6.2.6 Diagnosis of type 2 Diabetes Mellitus (T2D)

A blood sample was taken after an overnight fast. The diagnosis of T2D was confirmed according to the criteria of the American Diabetic Association, 2011 **[38]**. Participants were categorized as normal (fasting plasma glucose level < 5.6 mmol/L), prediabetic (fasting plasma glucose level between 5.6 - to 6.9 mmol/L) and diabetic (fasting plasma glucose level >7 mmol/L).

6.2.7 Measurement and Definition of Blood Pressure

Before blood pressure measurement, participants were asked to sit at rest for approximately five minutes. Blood pressure was measured using a slight touch ST-401 blood pressure monitor according to standard operating procedures. Diagnosis of HTN status was defined as per guidelines by the American Heart Association (AHA), 2020 (Table 6.1) [39]. Three categories of participants based on their BP are: (1) elevated blood pressure (systolic blood pressure (SBP):120–129 mm Hg and diastolic blood pressure (DBP) < 80 mm Hg), (2) HTN grade 1 (SBP:130–139 mm Hg and DBP:80–89 mm Hg), and (3) HTN grade 2 ≥ 140 mm Hg and ≥ 90. Based on these guidelines, the participants in the current study were categorized as normotensive or hypertensive according to these guidelines, and diagnosis of HTN was confirmed after evaluating the patients' medical history, symptoms, and prescribed medication for three months.

6.2.8 Sampling Procedure

The current case control study enrolled a total of 100 hypertensive individuals and 100 normotensive control individuals. Venous blood (5ml) was collected in EDTA tubes (anticoagulant) with written and informed consent. The samples were stored at -80 degrees celsius until further analysis. A blood sample was also taken after an overnight fast to measure the glucose levels. Furthermore, 24 participants were not included in the further analysis due to incomplete information. Genotyping was therefore done on 177 samples.

6.2.9 DNA Extraction

DNA extraction from blood samples was done using the QIAamp Blood Midi Kit (Spin Protocol) (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

6.2.10 DNA Quantification

Quantification of extracted DNA was done on the NanoDrop Spectrophotometer (Nanodrop Technologies) following the manufacturer's suggestion.

6.2.11 SNP Genotyping Analysis

Genotypes were determined using TaqMan SNP Validated Genotyping Assay (Applied Biosystems, Massachusetts, United States). It was based on the principle of the real-time polymerase chain reaction (PCR) method, where amplified products are accumulated during the exponential phase of the PCR cycle. Optimal thermal cycling conditions, fluorescence detection, and application-specific software allowed the cycle-by-cycle detection and increase in the number of amplified products in real-time. The TaqMan SNP genotyping technology used Taq polymerase's 5' nuclease activity to emit a fluorescence signal during PCR. The assay used two TaqMan probes for each SNP, one complementary to the wild-type allele and the other to the variant allele, with the sequence differences occurring solely at the SNP location.

6.2.12 SNP Genotyping Primer Selection

Two SNPs of *AGT* (rs2004776 & rs3789678) and *ACE* (rs1799752 & rs4344) were selected, and primers were designed as given in Table 6.1. The File Builder tool was then used to order primers from Applied Biosystems and synthesized according to the specifications. The TaqMan custom genotyping assay probes (x40) were assigned the labels VIC and FAM to the sequences below.

Table 6.1. SNP selection and primer design

Gene	Variant	Chr	Location	SNP Sequence	Assay ID
AGT	rs2004776	1q42.2	g.230712956	GGCTGAATGCTAAAGGTGAAGATGA**[C/T]** GGCTCATGCTCCTGTGGTGCCTGCC	**C-1985478_10**
	rs3789678		g.230713736	CCTTCCCTCCAGCCCCAATTCCTG**[C/T]** ACAAGCCCTGCTATTCCTCCTGATG	**C-27483307_10**
ACE	rs1799752	17q23.3	g.63488530	CCCATTTCTCTAGACCTGCTGCCT**[REF/-]** ATAC*AGT*CACTTTTATGTGGTTTC	**C-60538594B_20**
	rs4344		g.63489363	CTATGTCGGGCA*AGT*CACCATGGAT**[A/G]** GGGGAAGA*AGT*TAATAATCTTGTCC	**C-11942565_10**

6.2.13 Polymerase Chain Reaction Preparation

All samples were diluted to a final concentration of 10ng/µl and aliquoted individually (4.75µl) in each well in a 96 well plate. A PCR mastermix was prepared by adding 5 µL of TaqPath ProAmp Master Mix and 0.25 µL of 40X TaqMan SNP Genotyping Assay in a total volume of 10 µL, according to the manufacturer's instructions (Applied Biosystems). A volume of 5.25 µl of the master mix was added to each well, yielding a final volume of 10 µl (4.75 µl sample + 5.25µl master mix) in each well. A negative control of the same volume was also added with each PCR run. The Quant studio 5 (Applied Biosystems, Massachusetts, United States) was used for the PCR amplification. PCR parameters were as follows: Pre-read for 30s at 60°C (Hold); initial denaturation for 5 min at 95°C (Hold); denaturation for 5s at 95°C 40 cycles; annealing/extension for 30s at 60°C 40 cycles; post-read for 30s at 60°C (Hold). All samples were genotyped in duplicate. The results of the amplified PCR products were viewed using design and analysis software version 2.4.3.

6.2.14 Statistical Analysis

Clinical and anthropometric parameters were represented as mean ± standard deviation (SD). The continuous data were compared using a student's *t*-test and one-way analysis of variance (ANOVA). Bartlett's test for equal variance was used to obtain significance values for the corresponding differences in means. Allelic and genotypic frequencies were compared. The conformity of genotype distributions was evaluated using Hardy-Weinberg equilibrium. The genetic association with HTN was tested using the Chi-squared test and odds ratio with 95% confidence intervals and various inheritance models (dominant, recessive, and codominant) and logistic regression models enabling us to adjust for age and gender, BMI, and waist circumference (WC). Haploview software (Version 4.2; https://www.broadinstitute.org) was used

to investigate the linkage disequilibrium between various SNPs and haplotype disease association. P-values < 0.05 were described as a significant association of an SNP/variable with the disease. Statistical analysis was mainly performed using the STATA (Software for Statistics and Data Science; package SE).

6.3 Results

The demographic, clinical, and anthropometric parameters of 177 participants (87 normotensive controls and 90 hypertensive cases) are represented in Table 6.2. Concerning age, the hypertensive cohorts had a higher mean age of 55 years as compared to normotensive controls (<42), with the differences being significant in the total pooled ($p<0.001$), male ($p=0.003$), and female ($p<0.001$) groups. Hip circumference (HC), and waist-hip ratio, fasting glucose levels, SBP, DBP, were significantly higher ($p<0.05$) in hypertensive cases as compared to normotensive controls in the total pooled sample. The gender-specific analysis revealed that in females, mean values of BMI, WC, and WHR, SBP, DBP were significantly different ($p<0.05$) in cases vs controls but males, only SBP, and DBP were significantly different ($p<0.05$) between the respective two groups. Hypertensive males had a mean BMI (26.01 ± 4.67 kg/m²) in the overweight range, whereas the hypertensive females were obese with a mean BMI of 32.64 ± 7.70 kg/m². Of interest, fasting blood glucose levels were higher in hypertensive cases as compared to normotensive controls in both the total pooled and female groups; however, significant differences ($p=0.06$) were observed only for the female group. The mean values of fasting glucose levels among hypertensive males and females were 5.41mmol/L and 6.50mmol/L, respectively. The corresponding values for the normotensive control groups were 7.4mmol/L and 5.6mmmol/L, respectively. No significant differences were observed between hypertensive cases and normotensive controls for the male ($p=0.202$) group. Hypertensive females were categorized with blood glucose levels in the pre-diabetic range showing moderate significance ($p=0.059$) compared to the female normotensive control group.

Table 6.2 Comparison of various parameters (mean ± SD) in total pooled, male, and female hypertensive cases and normotensive controls.

Variable		Hypertensive cases N=90 (79 Female & 11 Male)	Normotensive controls N=87 (69 Female & 18 Male)	P-value Bartlett's test for equal variances	P-value *t*-test equality of means
Age (years)	Total	55.08 ± 11.34	42.03 ±11.56	0.736	<0.001*
	Male	59.36 ± 11.87	43.83 ± 13.16	0.723	0.003*
	Female	54.48 ± 11.21	41.57 ± 11.42	0.875	<0.001*
BMI (kg/m²)	Total	31.81 ± 7.70	28.20 ± 6.10	0.033*	<0.001*
	Male	26.01 ± 4.67	23.61 ± 4.88	0.882	0.207
	Female	32.64 ± 7.70	29.33 ± 5.86	0.023*	0.004*

Waist circumference (cm)	Total	101.69 ± 14.02	92.98 ± 14.50	0.753	<0.001*
	Male	92.31 ± 9.71	87.05 ± 13.05	0.324	0.263
	Female	103.03 ± 14.07	94.44 ± 14.56	0.773	<0.001*
Hip circumference (cm)	Total	113.78 ± 15.16	108.02 ± 13.32	0.234	0.008*
	Male	103.40 ± 4.91	96.88 ± 10.60	0.016*	0.068
	Female	115.26 ± 15.56	110.77 ± 12.52	0.069	0.058
WHR	Total	0.89 ± 0.09	0.86 ± 0.09	0.653	0.013*
	Male	0.89 ± 0.06	0.89 ± 0.07	0.628	0.831
	Female	0.89 ± 0.09	0.85 ± 0.10	0.733	0.005*
Fasting blood glucose (mmol/L)	Total	6.36 ± 2.83	6.01 ± 3.00	0.599	0.433
	Male	5.41 ± 1.41	7.42 ± 4.94	<0.001*	0.202
	Females	6.50 ± 2.96	5.66 ± 2.21	0.015*	0.059
SBP (mmHg)	Total	147.05 ± 22.92	120.93 ± 11.35	<0.001*	<0.001*
	Male	141.27 ± 26.27	123.52 ± 9.82	0.001*	0.017*
	Female	147.88 ± 22.48	120.29 ± 11.68	<0.001*	<0.001*
DBP (mmHg)	Total	89.72 ± 11.92	79.06 ± 9.68	0.055	<0.001*
	Male	87.84 ± 10.96	78.47 ± 9.95	0.737	0.027*
	Female	89.99 ± 12.10	79.20 ± 9.68	0.062	<0.001*

Legend: BMI – Body Mass Index, SBP – Systolic Blood Pressure, DBP – Diastolic Blood Pressure, WHR – Waist Hip Ratio. *P < 0.05 significant.

6.3.1 Genotyping and Allele Frequency

A univariate analysis was used to identify SNP association with the disease status, and the data were adjusted for age, gender, BMI, and waist circumference. The genotype distribution conformity was assessed using the Hardy-Weinberg equilibrium (HWE) and it was observed that genotypes in both cases and controls were in equilibrium. Genotypic and allelic frequencies in hypertensive cases and normotensive controls for the studied SNPs are given in Table 6.3. For the SNP rs2004776, no significant differences were observed for genotypic and allelic frequencies between hypertensive cases and normotensive controls (OR=0.7985 (0.536-1.188), p=0.266, $\chi 2$=1.236). Similar findings have been observed for the other two SNPs, the genotypic and allelic frequencies between hypertensive cases and normotensive controls were not significantly different for rs3789678 (OR=1.190 (0.790-1.791), p=0.405, $\chi 2$=0.6944) and rs4344 (OR=1.086 (0.7293-1.617), p=0.685, $\chi 2$=0.164). Univariate analysis demonstrated that the studied SNPs (rs2004776, rs3789678, and rs4344) were not associated with a higher risk of developing HTN. However, SNP rs1799752 polymorphism was not observed in the studied population, and therefore, further analysis of this SNP was not carried out.

Table 6.3. Genotypic Frequency and Allelic Frequency distribution for hypertensive cases and normotensive controls

Genotype/allele frequency	Hypertensive cases	Normotensive controls
	rs2004776	
CC	18 (20.0)	22 (25.3%)
CT	40 (44.4)	38 (43.7%)
TT	32 (35.5)	27 (31.0%)
C	42.2	47.1%
T	57.7	52.8%
Univariate analysis	OR=0.798 (0.53-1.18), p=0.2662, χ^2=1.236	
	rs3789678	
CC	39 (43.3)	35 (40.2)
CT	41 (45.5)	36 (41.3)
TT	10 (11.1)	16 (18.3)
C	66.1	60.9
T	33.9	39.0
Univariate analysis	OR=1.190 (0.79-1.79), p=0.4047, χ^2=0.6944	
	rs4344	
GG	22 (22.2)	17 (19.5)
GA	38 (42.2)	39 (44.8)
AA	32 (35.5)	31 (35.6)
G	43.3	42.5
A	56.6	57.4
Univariate analysis	OR=1.086 (0.729-1.617), p=0.6847, χ^2=0.1649	
	rs1799752	
Allele 1	99 (50)	99 (50)

Legend: OR – Odds Ratio.

6.3.2 Inheritance models and Univariate analysis

Various hypothetical inheritance models were used to analyse the association of each SNP with the disease status Table 6.4. For SNP rs2004776, under the dominant (OR=1.353 (0.667-2.746), p=0.401, aOR=1.199 (0.498-2.889), p=0.685), recessive (OR=1.226 (0.652-2.294), p=0.524, aOR=1.293 (0.602-2.776), p=0.508), and codominant (OR=1.031 (0.570-1.867), p=0.918, aOR=0.897 (0.437-1.844), p=0.769) inheritance models, the T allele did not confer any risk of developing HTN. The analysis of rs3789678 polymorphism also demonstrated no significant association of the risk allele with disease status, under dominant (OR=0.880 (0.484-1.600), p=0.676, aOR=0.667 (0.316-1.406), p=0.287), recessive (OR=0.554

(0.236-1.300), p=0.175, aOR=0.745 (0.263-2.111), p=0.580), and codominant (OR=1.185 (0.653-2.149), p=0.575, aOR=0.768 (0.361-1.634), p=0.494) inheritance models. In addition, the rs4344 polymorphism also did not show any significance under dominant (OR=0.85 (0.411-1.757), p=0.728, aOR=0.710 (0.282-1.786), p=0.468), recessive (OR=0.996 (0.538-1.844), p=0.992, aOR=1.133 (0.540-2.377), p=0.740), and codominant (OR=0.899 (0.496-1.630), p=0.727, aOR=0.722 (0.351-1.482), p=0.375) inheritance models.

Table 6.4. Inheritance models and univariate analysis

	Unadjusted OR (95% CI)	Unadjusted p	aOR (95% CI)	Adjusted p
		rs2004776		
CC vs CT+TT Dominant model	1.353 (0.667-2.746)	0.401	1.199 (0.498-2.889)	0.685
TT vs CT+CC Recessive model	1.226 (0.652-2.294)	0.524	1.293 (0.602-2.776)	0.508
CT vs CC+TT Codominant model	1.031 (0.570-1.867)	0.918	0.897 (0.437-1.844)	0.769
		rs3789678		
CC vs CT+TT Dominant model	0.880 (0.484-1.600)	0.676	0.667 (0.316-1.406)	0.287
TT vs CT+CC Recessive model	0.554 (0.236-1.300)	0.175	0.745 (0.263-2.111)	0.580
CT vs CC+TT Codominant model	1.185 (0.653-2.149)	0.575	0.768 (0.361-1.634)	0.494
		rs4344		
GG vs GA+AA Dominant model	0.85 (0.411-1.757)	0.728	0.710 (0.282-1.786)	0.468
AA vs GA+GG Recessive model	0.996 (0.538-1.844)	0.992	1.133 (0.540-2.377)	0.740
GA vs GG+AA Codominant model	0.899 (0.496-1.630)	0.727	0.722 (0.351-1.482)	0.375

Legend: aOR – Adjusted Odd's Ratio (adjusted for age, gender, BMI, and waist circumference), CI – Confidence Intervals

6.3.3 Multivariate analysis

Multivariate logistic regression was used to collectively analyze the associated risk of the polymorphisms (Table 6.5). As interpreted from the unadjusted odds ratio, for rs2004776, the presence of the T allele increases the risk of developing HTN 3-fold compared to the C allele (p=0.038, OR=3.437(1.070 – 11.036). However, odds ratios did not suggest any association after adjusting for the various factors (p=0.067, aOR=3.853(0.907 – 16.350). Conversely, for rs3789678, the presence of the T allele confers protection against the development of HTN (p=0.021, OR=0.226 (0.063 - 0.800), however after adjusting for various factors the association of rs3789678 did not remain significant. (p=0.066, aOR=0.232(0.049 – 1.101). The polymorphism rs4344 did not demonstrate an association with the risk of developing HTN in the multivariate regression model

Table 6.5. Multivariate logistic regression model of various SNPs associated with hypertension

Genotype	Unadjusted Odds ratio	Unadjusted P>|z|	Unadjusted 95% CI	Adjusted Odds ratio	Adjusted P>|z|	Adjusted 95% CI
			rs2004776			

CT	1.705	0.271	0.658 – 4.418	1.877	0.278	0.601 – 5.859
TT	3.437	0.038*	1.070 – 11.036	3.853	0.067	0.907 – 16.350
			rs3789678			
CT	0.647	0.311	0.278 - 1.502	0.412	0.091	0.147 – 1.151
TT	0.226	0.021*	0.063 - 0.800	0.232	0.066	0.049 – 1.101
			rs4344			
GA	0.831	0.654	0.370 - 1.866	0.633	0.331	0.217-1.671
AA	0.846	0.696	0.367 - 1.950	0.800	0.681	0.278-2.307

Legend: *p < 0.05 significant, aOR – Adjusted Odd's Ratio (adjusted for age, gender, BMI, and waist circumference), CI – Confidence Intervals

6.3.4 Haplotype analysis

To enhance the study power and investigate the polymorphisms' influence on the disease state, haplotype analysis was performed for rs2004776 and rs3789678. Results obtained showed that two polymorphisms are in strong linkage disequilibrium (LD) with a coefficient D prime > 0.80 (Table 6.6), as could be observed with the haplotype block structure (D' = 1.00) (Figure 6.1). Next, the haplotype frequencies were determined, as shown in Figure 6.2. The CT haplotype was found to be significantly prevalent among cases (0.235) as compared to normotensive controls (0.135) (p=0.011). However, the CC and TT haplotypes were not significantly different among hypertensive cases compared to normotensive controls (p > 0.05).

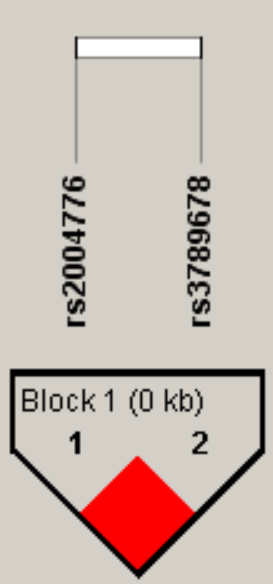

Figure 6.1. Linkage disequilibrium plot showing the interaction of rs2004776 and rs3789678

Table 6.6. Linkage disequilibrium analysis of rs2004776 and rs3789678

L1	L2	D'	LOD	r^2	CI low	CI hi	Distance	T-int
rs2004776	rs3789678	1.0	35.21	0.472	0.94	1.0	780	35.21

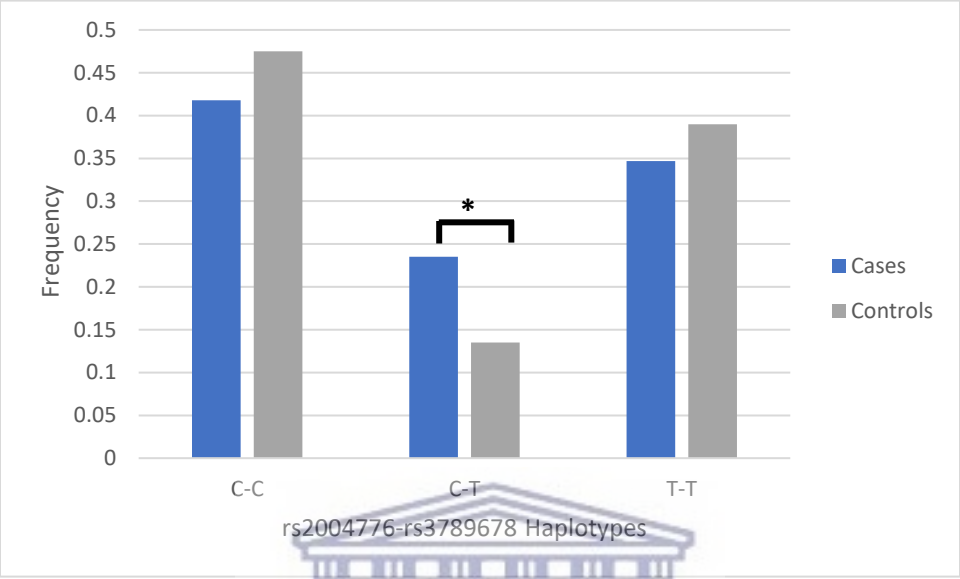

Figure 6.2. Haplotype frequency in hypertensive cases and normotensive controls.

6.4 Discussion

Hypertension is a major public health problem, particularly in the African region, contributing significantly to the increasing number of premature deaths in this region [40]. Due to the complex genetic diversity, and inheritance patterns in Africa, genetic studies carried out in these populations could reveal vital information about how genes influence human health and disease susceptibility [41]. Many studies have investigated the association of *AGT* and *ACE* SNPs with HTN in various populations; the contradictory outcomes of these studies necessitate the replication of SNP association studies in larger groups from multiple populations around the world. To date, no studies have reported the association of *ACE* or *AGT* polymorphisms with HTN in a Xhosa South African population. The present study is the first report to investigate the association of *AGT* polymorphism (rs2004776 and rs3789678) and *ACE* polymorphisms (rs1799752 and rs4344) with HTN in a Xhosa population of Eastern Cape, South Africa.

Inconsistent with other studies [11, 12, 42], the results of the present study also indicate that older age is a risk factor in the development of HTN. The mean age of hypertensive individuals was significantly higher than normotensive individuals in both males and females. It has been postulated that increased vascular resistance may contribute to higher HTN among older adults [43, 44].

Following earlier studies, findings of the current study strongly indicate that obesity could be a risk factor in the development of HTN in the studied population of the Eastern Cape. The present study observed significantly higher BMI in the hypertensive female as compared to the normotensive controls. [36]. Previous research has found that having a higher BMI is a major risk factor for HTN and that there is a linear association between BMI and HTN [11, 45, 46]. Obesity and visceral adiposity have also been shown to be positively linked with the renin-angiotensin system, which regulates blood pressure [47-49]. The mean waist circumference observed in the current study was significantly elevated in the hypertensive female group compared to the normotensive control group. Recently, Owolabi *et al.*, [50] suggested increasing the traditional waist circumference cut-off values (94cm and 80cm for males and females, respectively) to new cut-off values (≥95.25cm and ≥89.45cm for males and females, respectively) for sub-Saharan African populations. According to these new cut-off values, the total hypertensive, male hypertensive, and female hypertensive groups surpass the cut-off values; therefore, they are at greater risk of developing metabolic syndrome and HTN [51].

The mean hip circumference observed in the current study was significantly higher in the pooled hypertensive group compared to the normotensive controls; however, it was only marginally significant in the female and male hypertensive groups. Although hip circumference is not directly linked with HTN risk, it is used to calculate the waist-hip ratio, which is suggested to be a more accurate predictive tool for developing HTN than waist and hip circumferences alone [37].

Mean WHR observed in the current study was significantly higher in the total hypertensive and female hypertensive groups compared to their respective normotensive control groups. According to the WHO criteria, the cut-off values for WHR are ≥ 1.0, and ≥ 0.85 for males and females, respectively [52, 53] the total hypertensive, male hypertensive, and female hypertensive groups exceeded the cut-off threshold.

The gender-specific analysis revealed that BMI, waist circumference, and waist-hip ratio were significantly higher among hypertensive females than normotensive control females, and hip circumference showed a moderate significant difference between the two groups. However, the male group did not demonstrate significant differences for BMI, waist circumference, or waist-hip ratio between the hypertensive versus normotensive group.

The present study observed higher fasting blood glucose levels in the female hypertensive group than the normotensive control group; however, these differences were non-significant. Unexpectedly, mean blood glucose levels of the male normotensive control group were higher than that of the hypertensive group, however, the difference was not statistically significant, this needs further investigation. Nonetheless, according to the American Diabetic Association criteria [38], blood glucose levels of hypertensive female

falls within the pre-diabetic range. Similarly, another study found that diabetic individuals have nearly twice the risk of developing HTN compared to non-diabetic individuals [12]. Due to their overlapping metabolic pathways and risk factors, it is well known that there is significant commonality between HTN and diabetes [54]. Furthermore, a high BMI (overweight/obesity) is frequently linked to insulin resistance, which has substantial consequences for the development of type 2 diabetes mellitus (T2DM) [55, 56]. Obesity and T2DM are associated with an increased risk of death from cardiovascular illnesses [57, 58].

In the current study, we observed an association of T allele and TT genotype for the SNP rs2004776, which may increase an individual's susceptibility to developing HTN. Recently, another study done in an African population by [10] reported similar findings to the current study, where the TT genotype increased susceptibility to HTN. Studies in North American [59] and Indian [21] populations have previously supported the association of the T allele with HTN. In contrast to these studies, Li *et al*.[60] reported no association of rs2004776 with the development of HTN in a Chinese Han population, with a similar sample size as the current study. However, the study by Li *et al*.[60] investigated pregnancy-induced HTN, and thus the conclusions drawn may not be translational for this study. The comparison of the minor allele frequencies of this polymorphism in the current study with other global populations has revealed that the frequency of the minor allele (T-allele) of rs2004776 (52.5%) polymorphism is in line with that reported in another African population (46%) [10]. However, the minor allele frequency observed in the current study is drastically higher than the frequencies observed in two Asian populations (10-30%) [21, 60] and a North American population (22-24%) [59].

Functional studies investigating the rs2004776 polymorphism and HTN have confirmed the findings of the current case-control study and previous studies performed on North American and Indian populations. For example, Mopidevi *et al*., [61] utilised a knock-in approach in the hypoxanthine-guanine phosphoribosyltransferase locus to generate a transgenic mouse model containing the human renin gene and one of two haplotypes. The first haplotype (Haplotype 1) contained the TT genotype and was pro-hypertensive. The second haplotype (Haplotype 2) contained the CC genotype that did not have any significant effect on blood pressure. They showed that transgenic animals having Haplotype 1 had increased blood pressure compared to those containing Haplotype 2. They found that the mutant TT allele specifically caused the increase in blood pressure in the knockout animals. Furthermore, it was suggested that rs2004776 is in an open chromatin region, which implies the SNP can bind to transcription factors and modulate the expression of the *AGT* gene. They also suggested that the SNP has strong homology with the hepatocyte nuclear factor 3 binding site.

The present study also found an association of rs3789678 (*AGT*) and HTN in the study population. The presence of a rare allele (T allele) protects against developing a disease susceptibility. These findings are

supported by Gunda *et al.*, [21], they also reported a protective effect of this SNP in an Indian population. However, two studies performed in Chinese Han populations, have reported an association of rs3789678 polymorphism with an increased risk of developing HTN [60, 62]. However, the allele frequency in the study by Ji *et al.*, [63] deviated from the HWE. The minor allele (T-allele) frequency for rs3789678 reported in the present study is higher (38.9%) as compared to the frequencies observed in the four Asian populations (2-14%) [21, 60, 63, 64]. It was further suggested by Gunda *et al.*, [21] that the rs3789678 polymorphism in intron 1 of the *AGT* gene, located at the 350th nucleotide position, resulted in the development of a new exonic splicing enhancer site and the elimination of the exonic splicing silencer site, thereby enhancing the mRNA splicing process. Multifactor dimensionality reduction analysis was used by Gunda *et al.*, [21] to investigate the interaction among markers which revealed a strong synergistic effect between rs2004776 and rs3789678. This confirmed the present study's findings that demonstrated that both SNPs are in linkage disequilibrium, and a significant association of HTN with rs2004776–rs3789678 CT haplotype was determined.

The current study reported that there is no significant association of rs4344 polymorphism with a higher risk of developing HTN. Similar results were obtained in a Spanish population where no significant association was found between this SNP and HTN when investigating angiotensin-converting enzyme inhibitors-induced cough [65]. In contrast, two studies, one in a Mexican population and one in a Taiwanese population, have reported a significant association with the risk of developing HTN [22, 23]. The study by Chung *et al.*, included a large sample size with only young-onset HTN subjects and utilised SNP-chip technology to genotype their samples; the lack of controls questions the association found. Similar to the current study, Martínez-Rodríguez *et al.*, [23] utilised TaqMan genotyping assays and had a similar sample size. The minor allele frequency for rs4344 observed in the current study was higher than the previously observed frequencies in Mexican (42%) [23] and Spanish populations (39%) [65]. Limited studies have investigated the association of *ACE* polymorphism, rs4344, with HTN. The current study results need further investigation on a bigger sample group.

Limitations of the study include a small sample size; although meeting the power calculation requirements, a larger sample size would increase the accuracy and validity of results and associations found. The small sample size further limited association analysis between the genotypes and anthropometric parameters; therefore, the data were adjusted to correct for these confounding factors.

6.5 Conclusion

To conclude, the findings of the current study indicate that hypertensive females could be at higher risk of developing cardiovascular disease with significantly higher age, BMI, SBP, DBP, waist circumference, and waist-hip ratio compared to normotensive controls. The genotype distributions of *AGT* rs3789678 and

rs2004776 polymorphisms influenced the risk of developing HTN in the Xhosa South African population. It is deciphered that for rs3789678 polymorphism, TT genotype protects against the development of HTN and for rs2004776 polymorphism TT genotype confers a 3-fold risk of developing HTN. The present study also showed the two identified *AGT* polymorphisms were in strong linkage disequilibrium. The presence of the *ACE* rs4344 polymorphism did not confer any risk of developing HTN, while *ACE* I/D polymorphism (rs1799752) could not be detected in the studied population, and further analysis is needed on a broader group of the population. Additionally, more research on various other populations of South Africa and this population is warranted to corroborate the current findings. Other polymorphisms in the coding and promoter regions of the candidate genes analyzed should also be evaluated in various ethnic groups to determine their functional importance and impact on HTN. The discovery of these variants can aid in the understanding of genetic susceptibility factors in the population, allowing for better prevention and therapies.

6.6 Author Contributions:

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6.9 Conflict of Interest: The authors declare no conflict of interest.

6.10 Institutional Review Board Statement "The study was conducted following the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of SAMRC (SAMRC: EC028-8/2020) and Water Sisulu University (073/15) (Water Sisulu University (073/15).

6.11 Informed Consent Statement: "Informed consent was obtained from all subjects involved in the study."

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CHAPTER SEVEN

Original Research Manuscript

Although several beta 2-adrenergic receptors (*ADRB2*) gene SNPs have been found to be associated with HTN, the evidence for *ADRB2* gene association with HTN remains inconsistent. Thus, based on the results from **chapter 3**, (the predicted cluster 3) we performed the follow-up study where we sought to investigate the association between *ADRB2* polymorphisms (rs1042713 and rs1042714), and the risk of developing HTN in the Xhosa population of Mthatha, Eastern Cape (South Africa). This manuscript was designed to address objective five.

The chapter, as presented here, has been submitted in the journal of Molecular Genetics and Genomics (Impact Factor:3.3) [EMID:03e369b4986aad84]

My contribution:

Conceptualised and designed the study

Data curation

Analysed data and interpretation

Original draft preparation

CHAPTER 7: *ADRB2* Gene Polymorphisms as a Risk Factor for Hypertension Among an Indigenous Xhosa Population of Mthatha, Eastern Cape, South Africa

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Keyword: Hypertension; beta 2-adrenergic receptor gene; *ADRB2*; single-nucleotide polymorphism; genetic variation

Abstract

Background

Beta 2-adrenergic receptor gene (*ADRB2*) has long been implicated in the sympathetic nervous system and the regulation of blood pressure (BP). It has been reported that genetic variation in this receptor, leading to reduced vasodilation, could be responsible for increased peripheral resistance and blood pressure. Although several *ADRB2* single nucleotide polymorphisms (SNPs) have been found to be associated with hypertension (HTN), the evidence for *ADRB2* gene association with HTN is not conclusive. Thus, the purpose of this study was to investigate the association between two common *ADRB2* polymorphisms (rs1042713 and rs1042714), and the risk of developing HTN in an indigenous South African population from Mthatha Town of Eastern Cape, South Africa.

Methods

A total of 442 participants were recruited from the Xhosa tribe of Mthatha, Eastern Cape province in South Africa. The participants were divided into two groups, (i.) the control group (n = 163), and (ii.) the hypertensive group (n = 279). The HTN was defined based on American Heart guidelines with systolic and diastolic blood pressure ≥130/80 mm Hg. Peripheral blood samples were collected from all the subjects and DNA was extracted using a QIAamp DNA Blood Midi kit. Subsequently, the rs1042713 and rs1042714 polymorphism were analyzed by MassARRAY® System. Thereafter, the association between rs1042713 and rs1042714 in various genetic models (Genotypic, dominant, co-dominant, and allelic) and HTN was determined by logistic regression model analysis. Furthermore, the interaction between *ADRB2* (rs1042713 and selected various risk factors on HTN was investigated using the open-source multifactor dimensionality reduction (MDR).

Results

For the SNPs, rs1042713 and rs1042714, no significant differences were observed for genotypic and allelic frequencies between hypertensive cases and normotensive controls. Significant association between genetic variant (rs1042713) and HTN could also not be determined for various genetic models: genotypic AG (OR = 0.84, 95% CI = 0.50 - 1.38, p = 0.48), GG (OR = 0.66, 95% CI = 0.37 - 1.18, p = 0.16), dominant (OR = 0.76, 95% CI = 0.48 - 1.21, p = 0.26), recessive (OR = 0.73, 95% CI 0.44 - 1.21, p = 0.23), co-dominant models (OR = 0.99, 95% CI = 0.63 - 1.54, p = 0.95) and Allelic (OR = 0.76, 95% CI 0.58 – 1.01, p = 0.06). Furthermore, significant interaction was found between rs1042713 genotypes, BMI, and age on HTN in our study population.

Conclusion

The current study revealed that there is no significant association between the two genetic variants (rs1042714 and rs1042713) of the *ADRB2* gene with the risk of developing HTN. However, a potential synergistic effect occurred among rs1042713 genotypes, BMI, and age on HTN in our study population. However, future studies with a large sample size are required to further validate these findings and study the role of these *ADRB2* polymorphisms in the risk of developing HTN.

7.1. Introduction

Hypertension (HTN) is a significant public health concern and is a leading cause of cardiovascular-related mortality worldwide, affecting more than 1.4 billion individuals globally [1, 2]. In Sub-Saharan Africa, HTN is an escalating problem contributing to the rising number of premature deaths observed in the region. In particular, South Africa has been shown to have the heaviest burden of HTN with an estimated prevalence between 27–58% [3]. Despite an advanced understanding of pathogenesis, there is still a progressive rise in its prevalence. HTN is greatly considered as a complex genetic disorder caused by multiple susceptibility genes, regulated by gene-environment and gene interactions [4]. Therefore, many gene polymorphisms have been investigated as candidate determinants of the risk of developing HTN.

At the molecular level, the role of the beta 2-adrenergic receptor (*ADRB2*) gene in HTN has been extensively studied [5-8]. The *ADRB2* gene is a G-protein-coupled receptor located on chromosome 5q31-32, which plays a central role in BP regulation via sympathetic nervous system [9]. Accordingly, *ADRB2* regulates BP by controlling renin release, renal sodium excretion, and vascular resistance [10-12]. More than 80 polymorphisms in the ADRB2 gene have been identified in various populations [13]. Among the most important variants linked to increased BP are rs1042713 and rs1042714 polymorphisms.

Considering the crucial role of *ADRB2* gene polymorphisms in BP regulation, a large number of existing genetic studies in the broader literature have investigated the association between the *ADRB2* variants (rs1042713 and rs1042714) with HTN in different ethnicities [7, 14-16]. However, the results from the reported studies are still inconsistent. Despite the inconsistent results, a meta-analysis performed in 2010 by Lou *et al*, [17] found that the *ADRB2* rs1042713 polymorphism was associated with the risk of developing HTN in the Mixed African population. To the best of our knowledge, no follow-up study was performed to further confirm these findings in the indigenous African populations.

Importantly, our research group has actively explored this research niche within an indigenous South African population. In fact, previous data from our research group demonstrated a high prevalence (~75%) of HTN in the villages of Mthatha, Eastern Cape [18]. Furthermore, through a systematic approach review and computational insights, we further identified three clusters including the *ADRB2* gene in cluster 3 (co-

expression analysis), and further predicted possible mechanisms in which drugs can be targeted against HTN among individuals of African origin [19]. Therefore, this study sought to investigate the association between *ADRB2* polymorphisms (rs1042713 and rs1042714), and HTN in an indigenous South African population from Mthatha, Eastern Cape, South Africa. Furthermore, the study was extended to investigate single nucleotide polymorphism (SNP)-environment and sociodemographic interactions associated with the risk of HTN.

7.2. Materials and Methods

7.2.1. Ethical clearance

The research ethic for this study was approved by the Senate Research Committee of the University of the Western Cape (Ethics clearance number BM19/8/19), Water Sisulu University (073/15), and South African Medical Research Council (EC028-8/2020). The study was conducted according to the principles expressed in the Helsinki Declaration [20]. Written consent in both English and IsiXhosa was obtained from all participants.

7.2.2. Study design and patient selection

The present case-control study included participants (indigenous tribe) aged 18 years and above, from four districts (OR Tambo, Alfred Nzo, Chris Hani, Joe Gqabi), and five subdistricts, involving fourteen community health centres in Mthatha in the Eastern Cape Province of South Africa. HTN was defined as average SBP or DBP of ≥130/80 mmHg or using at least one class of antihypertensive medication following the American Heart Association guidelines. Pregnant patients and those unable to give consent were excluded to avoid inconvenience.

7.2.3. Data collection

The data records were collected using the World Health Organisation (WHO) STEPwise questionnaire which was uploaded onto the Research Electronic Data Capture (REDCap), a web-based application for building and managing online surveys and databases [34]. Data collected included information on sociodemographic factors (smoking, age, gender) and anthropometric measurements (body mass index, BMI, blood glucose reading). Ethnicity was defined as belonging to a social group with a common language (Xhosa), and cultural tradition.

7.2.4 BMI and Obesity Profiles

Body mass index (BMI) was obtained by dividing a person's weight in kilograms (Kg) by the square of the person's height in meters (m). Participants were either classified as normal (BMI ≤ 25Kg/m^2), overweight (BMI ≥ 25-29.9 Kg/m^2), or obese (BMI ≥ 30 Kg/m^2) according to the WHO standards [21].

7.2.5 Diagnosis of Diabetes Mellitus

A blood sample was taken after an overnight fast. The diagnosis of diabetes was confirmed according to the criteria of the American Diabetic Association, 2011 [22]. Participants were categorized as normal (fasting plasma glucose level < 5.6 mmol/L), prediabetic (fasting plasma glucose level between 5.6 - to 6.9 mmol/L), and diabetic (fasting plasma glucose level >7 mmol/L).

7.2.6 Sampling Procedure

The current study enrolled a total of 279 hypertensive individuals and 163 normotensive control individuals. Venous blood (5ml) was collected in EDTA tubes. The samples were stored at -80 degrees celsius until used. A blood sample was also taken after an overnight fast to measure the glucose levels.

7.2.7 Genomic DNA isolation

Peripheral blood samples were collected from all subjects and the DNA was extracted using a QIAamp DNA Blood Midi kit (Qiagen, Valencia, CA, USA) as per the manufacturers' instructions. DNA quantity and quality were determined using a Nano-Drop™ 2000/2000c UV/VIS Spectrophotometer (ThermoScientific™). Thereafter, the presence of *ADRB2* SNPs (rs1042713 and rs1042714) was analyzed using the MassARRAY® System (Agena Bioscience™). Genotypes of the selected SNP variants were determined for all the study participants as previously described by Masilela *et al*, [23].

7.2.8 Statistical analysis

Statistical analyses were performed using Stata/IC version 17.0 (Stata Corp, USA). The general characteristics of the participants were expressed as the actual number or percentage. The chi-square test was used to compare the differences in the distribution of baseline characteristics of categorical variables between hypertensive and normotensive (age, gender, smoking status, BMI, and blood glucose reading). Logistic regression analysis was used to further explore the relationships between independent variables (age, gender, smoking status, BMI, and blood glucose reading) and dependent variable (HTN). In addition, the potential effects of the *ADRB2* polymorphisms on HTN risk under various inheritance models (dominant, recessive, and co-dominant) were also examined using logistic regression analysis. The chi-square goodness of fit test was used to evaluate the significant difference for the genotypic distribution in the *ADRB2* (rs1042713 and rs1042714) between cases and controls. Various genetic models were also applied to study the association between the genetic variants and the risk of developing HTN as suggested by Thakkinstian *et al*. [24]. The probability of exposure given the outcomes (OR) was used to study the association of various genotypes with disease status. To indicate the precision of the effect, 95% CIs were calculated. Two-tailed statistical significance was evaluated by using a p-value of < 0.05. Interactions between *ADRB2* (rs1042713 and selected variables on HTN were detected using the open-source

multifactor dimensionality reduction (MDR) software package version 3.0.2. The best model of interaction was selected based on *p*-values <0.05.

7.3. Results

The distribution of baseline characteristics of categorical variables such as age, gender, smoking status, BMI, and blood glucose reading between hypertensive and normotensive individuals was adopted as previously reported by Sharma *et al*, [18] in our group. We included 279 participants with HTN as cases and 163 participants as normotensive. In table 7.1, we summarize the ORs with corresponding 95% CIs for the association of the risk factors (age, gender, smoking status, BMI, and blood glucose reading) with HTN using logistic regression analyses. Logistic regression analysis showed that older age (36 to ≥65) ($p = 0.007$ and $p < 0.001$ respectively), being male ($p = 0.034$), smoking habits ($p = 0.012$), obese ($p < 0.001$), and prediabetic/diabetic ($p = 0.031$) with the increasing risk of developing HTN.

Table 7.1 Associations of age, gender, BMI, smoking status, and blood glucose reading with the risk of hypertension.

Variable	Hypertensive	Non-hypertensive	Crude odds ratios (95% CI)	*p*-Value
Age (years)				
18-35 years	51	58	1.0*	
36-49 years	93	53	1.99 (1.20-3.31)	0.007
50-64 years	93	39	2.71 (1.59-4.60)	<0.001
≥65 years	42	13	3.67 (1.77-7.60)	<0.001
Gender				
Female	235	125	1.0*	
Male	44	38	1.69 (1.03-2.78)	0.034
Smoking Status				
No	13	18	1.0*	
Yes	263	141	0.39 (0.18-0.81)	0.012
Body Mass Index				
Normal	57	59	1.0*	
Overweight	65	40	1.68 (0.98-2.87)	0.057
Obese	156	51	3.16 (1.95-5.12)	<0.001
Blood glucose levels				
Normal - FBS < 5.6 mmol/L	163	96	1.0*	
Prediabetic - FBS > 5.6 – 6.9	59	26	0.73 (1.01-2.96)	0.065
Diabetic - FBS > 7 mmol/L	57	41	1.39 (2.11-5.45)	0.031

Abbreviations: BMI, body mass index; CI, confidence interval. Adjusted OR: [a] adjusted for age, gender, smoking status, BMI, and blood glucose reading. * Reference. [#] *p*-Value < 0.05 was considered statistically significant.

Table 7.2 shows the genotypic and allelic frequencies *ADRB2* variants (rs1042713 and rs1042714) in both case and controls. The frequencies of alleles and genotypes of rs1042713 and rs1042714 among our study population were distributed according to the Hardy-Weinberg equilibrium ($p > 0.05$). The observed rs1042713 genotypes were [homozygous AA (hypertensive, 41% and normotensive, 57%), heterozygous AG (hypertensive, 37% and normotensive, 44%) and homozygous genotype GG (hypertensive, 22% and

normotensive, 27%)]. Furthermore, their allele frequencies were [A allele (hypertensive, 59% and normotensive, 51%), G allele (hypertensive, 41% and normotensive, 49%)]. We further investigated the associations of the *ADRB2* variants (rs1042713 and rs1042714) with the risk of developing HTN using logistic regression analysis under all genetic models. In the univariate analysis, the *ADRB2* rs1042713 variant was not significantly associated with HTN under various inheritance models dominant (OR = 0.72, 95% CI = 0.47 - 1.10, p = 0.26), recessive (OR = 0.71, 95% CI 0.45 - 1.13, p = 0.15) and co-dominant models (OR = 0.95, 95% CI = 0.63 - 1.42, p = 0.82). After adjustments for age, gender, smoking status, BMI, and blood glucose reading, there was still no significant association observed in genotypic AG (OR = 0.84, 95% CI = 0.50 - 1.38, p = 0.48), GG (OR = 0.66, 95% CI = 0.37 - 1.18, p = 0.16), dominant (OR = 0.76, 95% CI = 0.48 - 1.21, p = 0.26), recessive (OR = 0.73, 95% CI 0.44 - 1.21, p = 0.23), co-dominant models (OR = 0.99, 95% CI = 0.63 - 1.54, p = 0.95) and Allelic (OR = 0.76, 95% CI 0.58 – 1.01, p = 0.06). Furthermore, low frequencies of rs1042714 G allele (2%) in our study population was observed when compared with the C allele (98%) and precluded a proper logistic regression analysis.

Table 7.2. Association of the *ADRB2* variants (rs1042713 and rs1042714) in correlation with risk of developing hypertension.

SNP	Hypertensive (*n*; %)	Normotensive (*n*; %)	Crude odds ratios (95% CI)	*p*-Value	Adjusted odds ratios (95% CI)	*p*-Value
rs1042713						
			Genotypic			
AA	(102; 38)	(48; 31)	1.0*		1.0*	
AG	(107; 40)	(64; 41)	0.78 (0.49 - 1.24)	0.31	0.84 (0.50 - 1.38) [a]	0.48
GG	(59; 22)	(44; 28)	0.63 (0.37 - 1.06)	0.08	0.66 (0.37 - 1.18)	0.16
			Dominant			
AA	(102; 38)	(48; 31)	1.0*		1.0*	
AG + GG	(166; 62)	(108; 69)	0.72 (0.47 - 1.10)	0.13	0.76 (0.48 - 1.21) [a]	0.26
			Recessive			
AA +AG	(209; 78)	(112; 72)	1.0*		1.0*	
GG	(59; 22)	(44; 28)	0.71 (0.45 - 1.13)	0.15	0.73 (0.44 - 1.21) [a]	0.23
			Co-dominant			
AA + GG	(161; 60)	(92; 40)	1.0*		1.0*	
AG	(107; 40)	(64; 60)	0.95 (0.63 - 1.42)	0.82	0.99 (0.63 - 1.54) [a]	0.95
			Allelic			
A	(311; 58)	(160; 51)	1.0*			
G	(225; 42)	(152; 49)	0.76 (0.58 - 1.01)	0.06		
rs1042714						
			Genotypic			
GG	(4; 1)	(2; 2)	1.0*		1.0*	
CG	(2; 1)	(2; 2)	0.50 (0.04 – 6.68)	0.60	0.07 (0.003 – 1.59) a	0.09
CC	(267; 98)	(154; 96)	0.86 (0.16 – 4.79)	0.87	0.18 (0.18 - 1.89) a	0.15
			Dominant			
GG	(4; 1)	(3; 2)	1.0*		1.0*	
CG + CC	(269; 99)	(195; 98)	0.86 (0.16 – 4.76)	0.87	0.18 (0.02 - 1.89)	0.16
			Recessive			
GG +CG	(6; 2)	(4; 3)	1.0*		1.0*	
CC	(267; 98)	(154; 97)	1.16 (0.32- 4.16)	0.82	0.66(0.14 - 2.97)	0.59
			Co-dominant			
GG + CC	(271; 99)	(156; 99)	1.0*		1.0*	

CG	(2; 1)	(2; 1)	0.57 (0.08 – 4.13)	0.58	0.39 (0.05 – 2.99) a	0.37
			Allelic			
G	(10; 2)	(6; 2)	1.0*			
C	(536; 98)	(310; 98)	1.03 (0.37 - 2.88)	0.94		

Abbreviations: *ADBR2*, beta 2-adrenergic receptor gene; CI, confidence interval. [a] Adjusted OR: adjusted for age, gender, smoking status, BMI, and blood glucose reading. *Reference. *p*-Value > 0.05 was considered statistically not significant.

To explore the interaction of environmental factors and *ADBR2* polymorphism on HTN, SNP-environmental interactions between the genotypes of *ADBR2* rs1042713 and selected environmental factors on HTN were analysed using multifactor dimensionality reduction (MDR). As shown in table 7.3, the combination of rs1042713, age, and BMI had a high cross-validation consistency (CVC) score (10/10), and the model was significantly associated ($p < 0.0001$). On the other hand, the combination of age, and BMI (CVC=7/10) was significantly associated with the incidence of HTN ($p = 0.0004$). These findings indicate that significant interaction occurred among rs1042713 genotypes, BMI, and age on HTN. Other possible interactions are shown in **Figure 7.1.**

Table 7.3. Interaction models between *ADBR2* rs1042713 and selected environmental factors.

Interaction models	Training Score	Testing Score	CVC	p-value
Age	4.6486	3.8398	8/10	0.0002
Age, BMI	5.7898	3.9976	7/10	0.0004
rs1042713, Age, BMI	7.3389	3.3720	10/10	<0.0001

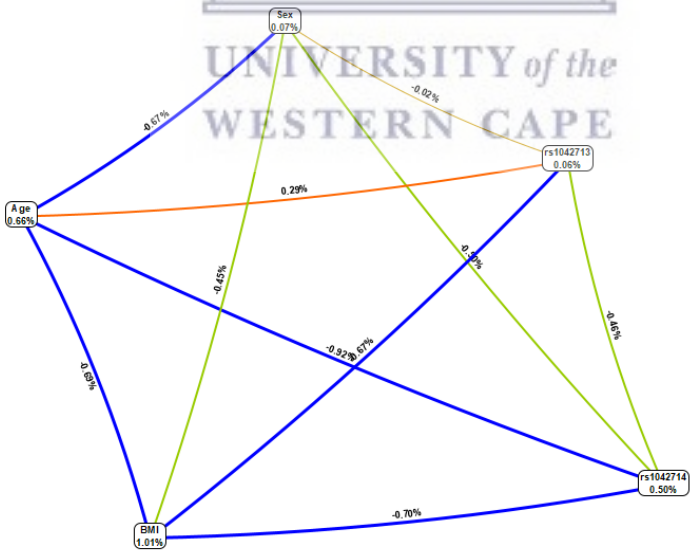

Figure 7.1: multifactor dimensionality reduction combined attribute network showing all possible interactions between *ADBR2* polymorphisms and selected environmental factors. Various interactions indicated by different colours between the genotypes of *ADBR2* rs1042713 and selected environmental factors were observed, and each colour represents a possible interaction. The width of the line indicates the strength of the interaction, whereas the thin lines represent weak interactions. Figures < 1 and thin lines

represent weak interactions. The strongest interactions are represented by figures ≥1 and thick lines. The image was generated using the open-source MDR software package version 3.0.2.

7.4. Discussion

Several studies have demonstrated that genetic variations of *ADRB2* are associated with the risk of developing HTN [25, 26]. Although numerous SNPs in the *ADRB2* gene have been detected in various ethnicities, the two most common SNPs (rs1042713 and rs1042714) have been reported to be strongly associated with HTN [13, 17]. However, the results remain inconsistent. The presence of both rs1042713 and rs1042714 is well known to downregulate the protein expression of *ADRB2* in hypertensive individuals and cause elevated BP [13]. Recently from our research group, we conducted a systematic approach review and computational insights that identified three clusters including the *ADRB2* gene in cluster 3 (co-expression analysis), and further predicted possible mechanisms in which drugs can be targeted against HTN among individuals of African origin [19]. Therefore the current study aim to evaluate the association between HTN and two common SNPs in the *ADRB2* gene (rs1042713 and rs1042714) in the indigenous South African population of Mthatha, Eastern Cape, South Africa.

In the indigenous South African population of Mthatha, we were not able to find an association in both rs1042714 and rs1042713 polymorphisms with the risk of developing HTN. Consistent with our findings, a study by Candy *et al*., [27] and Xie *et al*, [28] also failed to find any significant association between *ADRB2* polymorphisms and HTN in black South African and black Americans, respectively. In contrast, a study by Kotanko *et al*, [29] has reported that the rs1042713 variant is associated with HTN in African Caribbeans. Similar findings were also found in a study conducted by Tang *et al*. [30] in the mixed African population. Furthermore, a meta-analysis by Lou *et al*, [17] also found that the *ADRB2* rs1042713 polymorphism was associated with the risk of developing HTN in the Mixed African population. Importantly, the present study as well as the reference studies [27-30] were composed of relatively small sample sizes. Therefore, our results should be interpreted with caution [44], thus, the findings made remain to be explored in future studies with larger sample sizes. In addition, our negative results might be partially attributed to the complex genetic differences and the different environmental factors related to our study population. Therefore, heterogeneity of alleles frequencies among the ethnicities might be the main point of the different findings in other association studies.

Although no association was established between rs1042713 polymorphisms and the risk of developing HTN, a significant interaction was also reported among rs1042713, BMI, and age on HTN risk. These findings suggested that obesity and age might have an influence between genetic factors and the risk of developing HTN in the indigenous Xhosa population of Mthatha. This might be also related to the living habits and environments of our study population. In agreement with our findings in the previous studies

reported by Lou *et al*, [31] and Pereira *et al*, [32] also demonstrated an interaction between *ADRB2* rs1042713 and BMI with the risk of developing HTN in China and Brazil, respectively.

7.4.1 Limitations of This Study

It is noteworthy to mention that several limitations need to be considered in interpreting the findings of our study. (1), the sample size of our study was relatively small. In molecular epidemiological studies, small-sample size research is very likely to have random errors and bias, resulting in unreliable results [33]. Therefore, our results should be interpreted with caution until more studies on these underrepresented groups accumulate [34]. However, this will be a stepping-stone to larger ongoing clinical and genetic studies of HTN in South African population (2), This study used a cross-sectional design, which precludes causal inference. (3), our participants were recruited from only one ethnic group (Xhosa population of Mthatha, Eastern Cape) and our findings are generalised to the included study population and individuals residing in similar settings in the province. (4), due to the questionnaire used to collect personal exposure information (such as smoking and drinking), the responses might be influenced by the participant's memory, so recall bias cannot be excluded.

7.4.2 Strengths of This Study

Despite these limitations, to the best of our knowledge, this is the first study to evaluate the association between HTN and two common SNPs in the *ADRB2* gene (rs1042713 and rs1042714) in the indigenous South African population of Mthatha, Eastern Cape, South Africa., and further explore SNP-environment interaction, which opens the door for future research. Furthermore, our findings have begun to address the scarcity of *ADRB2* gene information in relation to HTN in Eastern Cape province of South Africa, although considerable work remains in sampling more broadly across the country. While the sample size of the present study was relatively small, it is often better to test a new research hypothesis on a small number of participants first. This helps to avoid spending too many resources such as time and financial costs, on finding an association between risk factors and the disease when there really is no association [35]. This indicates that there is an urgent need to carefully plan African-specific studies with large sample sizes to be able to draw conclusions on the association between the *ADRB2* gene (rs1042713 and rs1042714) and HTN.

7.5. Conclusion

In the current study, we were not able to find an association in both rs1042714 and rs1042713 polymorphisms with the risk of developing HTN. However, a potential synergistic effect occurred among rs1042713 genotypes, BMI, and age on HTN in our study population. Thus, future studies with a large sample size are required to further validate these findings and study the role of these *ADRB2* polymorphisms in the mechanism of HTN.

7.6. Authors Contributors

S.E.M and R.J conceptualised and designed the study. S.E.M acquired the data. S.E.M, C.M. J.R.S., and S.S. analysed and interpreted the data and drafted the manuscript. B.M., S.N, S.E.M, S.S, T.A, L.M, J.R.S, M.B., and R.J. were involved in the critical revision of the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

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7.9. Conflicts of Interest

The authors declare there are no conflicts of interest.

7.8. References

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CHAPTER EIGHT

Original Research Manuscript

The narrative review reported in chapter four evidenced that there was no significant association between *MTHFR* (rs1801133) and the development of HTN in an African population. Based on these results, we performed the follow-up study in our study population to further confirm these findings. Thus, this chapter sought to investigate the rs1801133 in *MTHFR* gene for its association with HTN among South African individuals in the Xhosa ethnic group. This manuscript was designed to address objective five.

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My contributions:

Conceptualised and designed the study

Collected and analyzed the data

Wrote the paper

CHAPTER 8: The Association of *MTHFR* (rs1801133) with Hypertension in an Indigenous South African population

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Keyword: Hypertension; methylenetetrahydrofolate reductase gene; *MTHFR*; single-nucleotide polymorphism; Africa; genetic variation

Abstract

Aims: The current study sought to investigate the association between the methylenetetrahydrofolate reductase (*MTHFR*) variant (rs1801133) and the risk of developing hypertension (HTN) in an indigenous South African population.

Methods: A total of 442 participants (hypertensive, *n* = 279 and non-hypertensive, *n* = 163) from the indigenous tribe residing in Mthatha, Eastern Cape (South Africa) were recruited. HTN was defined as a systolic (SBP) and diastolic blood pressure (DBP) of ≥130/80 mmHg following American Heart Association guidelines. The genotyping of *MTHFR* (rs1801133) was assessed using MassARRAY® System. Thereafter, the association between rs1801133 in various genetic models and HTN was determined by logistic regression model analysis. Furthermore, the interaction between rs1801133 and selected risk factors on HTN was performed using the open-source multifactor dimensionality reduction (MDR)

Results: The low frequency of the T allele (5%) was also observed when compared with the C allele (95%) in both cases and controls. After adjusting for confounding factors (gender, smoking status, BMI, and blood glucose levels), there were no significant associations were observed between rs1801133 and the risk of HTN in all genetic models: genotypic (OR 0.75, 95% CI 0.29 - 1.95, p = 0.56), dominant (OR 0.86, 95% CI 0.35 - 2.16, p = 0.75), co-dominant (OR 1.33, 95% CI 0.51 - 3.48, p = 0.55) and allelic (OR 0.80, 95% CI 0.49 - 1.62, p = 0.70) in logistic regression analysis. However, a significant interaction was reported among rs1801133, age, and gender (p <0.0001) with the risk of HTN.

Conclusion: The present study reports on the lack of association between MTHFR (rs1801133) and the risk of HTN in an indigenous South African tribe. However, an interaction between the gender, age, and rs1801133 was observed. Thus, future studies with a large sample size are required to further validate these findings.

8.1. Introduction

Hypertension (HTN) is a multifactorial disorder with genetic, environmental, and demographic factors contributing to its prevalence and progression. Over the past years, numerous researchers have uncovered the significant role of inter-individual genetic variations in the development, progression, and control of the disease [1-3]. Indeed, genome-wide association studies have successfully identified several genetic variants linked to the development and the progression of HTN [4, 5], including methylenetetrahydrofolate reductase (*MTHFR*) gene variants, with rs1801133 being the most common and widely studied variant located in this gene [6, 7]. However, their role in the pathophysiology of HTN particularly among individuals of African descent remains unclear [8, 9].

The *MTHFR* gene encodes methylenetetrahydrofolate reductase, a key regulatory enzyme in folate and homocysteine (Hcy) metabolism, and in the production of nitric oxide, a potent vasodilator that is important in blood pressure regulation [10, 11]. The rs1801133 polymorphism (677 C > T) is found on exon 4 of the *MTHFR* gene. The consequence of this polymorphism is a change of amino acid 222 from alanine to valine, leading to a decrease in *MTHFR* activity and an increase in plasma Hcy levels [12, 13]. Literature suggests that elevated plasma concentration of Hcy may injure the vascular endothelium, resulting in HTN [12, 13]. While other studies have shown that the *MTHFR* variant rs1801133 was associated with HTN in different study populations [14, 15].

Furthermore, these studies established that that carriers of rs1801133 present with high levels of plasma Hcy and are more likely to develop HTN [15, 16]. Whereas a reduction in *MTHFR* activity was linked with the loss of riboflavin, a cofactor associated with a decrease of Hcy among carriers of the *MTHFR* variant [16-18]. Most importantly, accumulative studies have suggested that *MTHFR* (rs1801133) polymorphism could be an independent risk factor for HTN in different ethnic groups [19-21], and it has been associated with severe diastolic HTN in pregnant women [22]. Even so, conflicting results have been obtained, particularly among African population studies [9, 20, 23, 24]. Thus, several authors have emphasized the need to conduct more African studies to further validate the role of *MTHFR* polymorphisms in the pathogenesis of HTN [9, 24, 25].

Undoubtedly, HTN, as a chronic medical condition, has contributed significantly to the rapid rise in global premature deaths, especially among Black African adults [26, 27]. Moreover, the devastating outcome has been the limited data currently informing on the incidence and pathological mechanisms driving the onset and progression of this medical condition among black African adults [28]. Studying the genetics of HTN can revolutionize our understanding of disease pathophysiology and the discovery of effective treatment strategies. Importantly, our research group has actively explored this research niche within an indigenous South African population. Our group has demonstrated a high prevalence (~75%) of HTN in the village of

Mthatha, Eastern Cape [26, 27]. We further conducted a narrative synthesis of the literature that predicted a possible association between *MTHFR* rs1801133 and the development of HTN among individuals of African origin [29]. Our findings highlighted the necessity of performing genomic studies on indigenous African individuals, as very few studies have addressed this research niche in a purely black African population. Thus, this study aimed to investigate the association between *MTHFR* (rs1801133) and HTN in the indigenous South African population of Mthatha in the Eastern Cape province of South Africa. Additionally, study aimed to explore the interaction between single nucleotide polymorphism (SNP) and sociodemographic as well as their interaction with the risk of HTN.

8.2. Material and methods

8.2.1 Ethical clearance

The ethical approval for this study was provided by the Senate Research Committee of the University of the Western Cape (Ethics clearance number BM19/8/19), Water Sisulu University (073/15), and the South African Medical Research Council (EC028-8/2020). The study conforms to the ethical guidelines of the Declaration of Helsinki [30]. Written informed consent in both English and IsiXhosa was obtained from all participants of the present study.

8.2.2 Power and sample size estimation

The appropriate sample and power size were estimated using the Power and Sample Size Program (PS version 3.1.2). The study initially planned to recruit approximately 430 participants (1:1 ratio). "Previous data indicate that the probability of exposure among controls is 0,75. If the true odds ratio for disease in exposed subjects relative to unexposed subjects is 2, we will need to study 215 case patients and 215 control patients to be able to reject the null hypothesis that this odds ratio equals 1 with a probability (power) 0,8. The Type I error probability associated with this test of this null hypothesis is 0,05 [31, 32]".

8.2.3 Study design and patient selection

A total of 442 participants belonging to the indigenous Xhosa tribe in the Eastern Cape province of South Africa, aged 18 years and above were recruited from four districts (OR Tambo, Alfred Nzo, Chris Hani, and Joe Gqabi). Participants were divided into two groups (i.) the non-hypertensive group (n = 163), and (ii.) hypertensive group (n = 279). HTN was defined as average SBP or DBP of ≥130/80 mmHg or using at least one class of antihypertensive medication following the American Heart Association guidelines (AHA) [33]. Blood pressure values were measured 3 consecutive times, after 10 min of seated rest before the first measurement and 5 min intervals between each measurement with a manual aneroid sphygmomanometer. SBP and DBP were determined by the first and the fifth Korotkoff sounds. The

average of three consecutive measurements to the nearest 2 mmHg was recorded, with a time interval of at least two minutes. Participants were selected in general consultation in the same centres.

8.2.4 Data collection and Sampling Procedure

The data records were collected using the World Health Organisation (WHO) STEPwise questionnaire which was uploaded onto the Research Electronic Data Capture (REDCap), a web-based application for building and managing online surveys and databases. Data collected included information on environmental or sociodemographic factors (smoking, age, gender), anthropometric measurements (body mass index, BMI), and blood glucose levels. All selected variables were defined as previously described by Sharma *et al*, (2021) [26] Venous blood (5ml) was collected in EDTA tubes. The samples were stored at -80 degrees celsius until used. A blood sample was also taken after an overnight fast to measure the glucose levels.

8.2.5 Genomic DNA isolation and genotyping

Peripheral blood samples were collected from all subjects and the DNA was extracted using a QIAamp DNA Blood Midi kit (Qiagen, Valencia, CA, USA) as per the manufacturers' instructions. DNA quantity and quality were determined using a Nano-Drop™ 2000/2000c UV/VIS Spectrophotometer (ThermoScientific™). Thereafter, the presence of *MTHFR* (rs1801133) was analyzed using MassARRAY® System (Agena Bioscience™).

8.2.6 Statistical analysis

Statistical analyses were performed using Stata/IC version 17.0 (Stata Corp, USA). The general characteristics of the participants were expressed as frequency (percentages). A chi-square test (non-parametric) was used to evaluate the genotypic distribution of the *MTHFR* (rs1801133) variant. Logistic regression analysis was used to examine the potential effects of the selected SNP (rs1801133) on the risk of developing HTN under various genetic models (genotypic, dominant, recessive, co-dominant, and allelic models) [6]. A method suggested by Thakkinstian *et al*. [34] was used to select the most appropriate genetic model. Multivariate regression analysis was carried out between rs1801133 variant and independent variables such as age, gender, smoking status, BMI and blood glucose levels. The probability of exposure given the outcomes (OR) and their 95% confidence intervals (CI) was used to present the association between the SNP and the risk of HTN. A two-tailed statistical significance was evaluated by using a *p*-value of < 0.05. Interactions between *MTHFR* (rs1801133) and selected environmental factors on HTN were detected using the open-source multifactor dimensionality reduction (MDR) software package version 3.0.2. Minor allele frequency (MAF) and Hardy–Weinberg equilibrium (HWE) tests were calculated using Genetic Analysis in Excel (GenAIEx) Version 6.5, where a p-value < 0.05 indicated a deviation from the Hardy Weinberg principle.

8.3. Results

8.3.1. General Characteristics of the Study Cohort

The present study sampled 442 individuals over the age of 18 years, of whom 19% (*n* = 82) were males and 81% (*n* = 360) were females. The mean for age was 46.96 ± 14.55 years. The distribution of baseline characteristics of categorical variables (age, gender, smoking status, BMI, and blood glucose levels) is summarized in **Table 8.1**.

Table 8.1: General characteristics of the study cohort

Variable	Total *n* = 442, *n* (%)	Hypertensive *n* = 279, *n* (%)	Non-hypertensive *n* = 163, *n* (%)	*p*-values
Age (years)				<0.001
18-35 years	109 (25)	51 (18)	58 (36)	
36-49 years	146 (33)	93 (33)	53 (33)	
50-64 years	132 (30)	93 (33)	39 (24)	
≥65 years	55 (12)	42 (16)	13 (7)	
Mean age (years)				
18-35 years	28.83 ± 4.45	28.90 ± 4.45	28.77 ± 4.51	
36-49 years	42.55 ± 3.96	42.64 ± 3.83	42.39 ± 4.20	
50-64 years	56.21 ± 3.65	56.07 ± 3.67	56.56 ± 3.62	
≥65 years	72.38 ± 5.60	72.61 ± 5.84	71.61 ± 4.89	
Gender				0.035
Female	360 (81)	235 (84)	125 (77)	
Male	82 (19)	44 (16)	38 (23)	
Smoking Status				0.010
Yes	31 (7)	13 (5)	18 (11)	
No	404 (93)	263 (95)	141 (89)	
Body Mass Index				<0.001
Normal	116 (27)	57 (20)	59 (41)	
Overweight	105 (25)	65 (23)	40 (25)	
Obese	207 (48)	156 (57)	51 (34)	
SBP (mm Hg)	130.79 ± 20.59	140.02 ± 18.93	113.72 ± 9.82	<0.001
DBP (mm Hg)	83.46 ± 11.56	89.24 ± 9.74	72.78 ± 5.42	<0.001
Blood glucose levels				0.034
Normal - FBS < 5.6 mmol/L	259 (59)	163 (59)	96 (59)	
Prediabetic - FBS > 5.6 – 6.9	85 (19)	59 (21)	26 (16)	
Diabetic - FBS > 7 mmol/L	98 (22)	57 (20)	41 (25)	

Chi-square tests of association were conducted; significance is determined at $p < 0.05$ (overall, for a given parameter).

3.2. Correlation between Covariates and Hypertension Susceptibility

In **table 8.2,** summarize the ORs with corresponding 95% CIs for the association of environmental factors (age, gender, smoking status, BMI, and blood glucose levels) with HTN using univariate analyses. In the unadjusted model, the logistic regression analysis showed a significant association with older age (36 to

≥65) (p = 0.007 and p < 0.001 respectively), being male (p = 0.034), smoking habits (p = 0.012), obese (p < 0.001), and prediabetic/diabetic (p = 0.031) with the increasing risk of developing HTN.

Table 8.2. Associations of age, gender, BMI, smoking status, and blood glucose levels with the risk of developing HTN.

Variable	Hypertensive	Non-hypertensive	Crude odds ratios (95% CI)	*p*-Value
Age (years)				
18-35 years	51	58	1.0*	
36-49 years	93	53	1.99 (1.20-3.31)	0.007
50-64 years	93	39	2.71 (1.59-4.60)	<0.001
≥65 years	42	13	3.67 (1.77-7.60)	<0.001
Gender				
Female	235	125	1.0*	
Male	44	38	1.69 (1.03-2.78)	0.034
Smoking Status				
No	13	18	1.0*	
Yes	263	141	0.39 (0.18-0.81)	0.012
Body Mass Index				
Normal	57	59	1.0*	
Overweight	65	40	1.68 (0.98-2.87)	0.057
Obese	156	51	3.16 (1.95-5.12)	<0.001
Blood glucose levels				
Normal - FBS < 5.6 mmol/L	163	96	1.0*	
Prediabetic - FBS > 5.6 – 6.9	59	26	0.73 (1.01-2.96)	0.065
Diabetic - FBS > 7 mmol/L	57	41	1.39 (2.11-5.45)	0.031

Abbreviations: BMI, body mass index; CI, confidence interval. *Reference. p-Value < 0.05 was considered statistically significant.

8.3.3 Association between *MTHFR* (rs1801133) Polymorphism and Hypertension Susceptibility

Table 8.3 shows the genotypic and allelic frequencies for the *MTHFR* (rs1801133) variant and the risk of developing HTN under genotypic, dominant, recessive, co-dominant, and allelic. The most frequently observed genotype was the homozygous CC (hypertensive, 91% and non-hypertensive, 89%) followed by heterozygous CT (hypertensive, 8% and non-hypertensive, 11%). The homozygous genotype TT had the lowest frequency in the studied population (hypertensive, 1% and non-hypertensive, 0%). Furthermore, the low frequency of the *MTHFR* (rs1801133) T allele (5%) was also observed in both hypertensive and non-hypertensive individuals when compared with the C allele (95%). After testing for Hardy-Weinberg principle using X^2 and p < 0.05, all alleles and genotypes were in agreement with the hypothesis (p = 0.069).

In the univariate analysis, the *MTHFR* rs1801133 variant was not significantly associated with HTN under various inheritance models [genotypic (OR = 0.75, 95% CI = 0.29 - 1.95, p = 0.56), dominant (OR = 0.86, 95% CI = 0.35 - 2.16, p = 0.75), recessive (*N/A*), co-dominant (OR = 1.33, 95% CI = 1.51 - 3.48, p = 0.55) and allelic models (OR = 0.80, 95% CI = 0.49 - 1.62, p = 0.70)]. After adjusting for confounding factors

such as age, gender, smoking status, BMI, and blood glucose levels, the direction of association did not change (p >0.05).

Table 8.3. Association of the *MTHFR* (rs1801133) polymorphism with risk of developing HTN.

SNP	Hypertensive (*n*; %)	Non-hypertensive (*n*; %)	Crude odds ratios (95% CI)	*p*-Value	Adjusted odds ratios (95% CI)	*p*-Value
rs1801133						
(HWE, p = 0.069)						
			Genotypic			
CC	(254; 91)	(144; 89)	1.0*		1.0*	
CT	(21; 8)	(18; 11)	0.64 (0.33 - 1.25)	0.19	0.75 (0.29 - 1.95) [a]	0.56
TT	(3; 1)	(0; 0)	-	-	-	-
			Dominant			
CC	(254; 91)	(144; 89)	1.0*		1.0*	
CT + TT	(24; 9)	(18; 11)	0.75 (0.40 - 1.43)	0.34	0.86 (0.35 - 2.16) [a]	0.75
			Recessive			
CC +CT	(275; 99)	(162; 100)	1.0*		1.0*	
TT	(3; 1)	(0; 0)	-	-	-	-
			Co-dominant			
CC + TT	(18; 8)	(22; 11)	1.0*		1.0*	
CT	(144; 92)	(256; 89)	1.48 (0.72 - 2.84)	0.24	1.33 (0.51 - 3.48) [a]	0.55
			Allelic			
C	(529; 95)	(306; 95)	1.0*			
T	(27; 5)	(18; 5)	0.86 (0.47 - 1.60)	0.65		

Abbreviations: **HWE,** Hardy-Weinberg Equilibrium; *MTHFR*, methylenetetrahydrofolate reductase; BMI, body mass index; CI, confidence interval. Adjusted OR: [a] adjusted for age, gender, smoking status, BMI and blood glucose levels. *Reference. *p*-Value > 0.05 was considered statistically not significant.

Epistatic interactions between the genotypes of *MTHFR* (rs1801133) and selected variables (age, gender, smoking status, BMI, and blood glucose levels) were assessed using multifactor dimensionality reduction (MDR) as presented in **Table 8.4**. The combination of age and rs1801133 had a high CVC score (9/10), however; the model was not significantly associated with HTN (p = 0.054). On the other hand, the combination of age, rs1801133, and gender (CVC=7/10) showed a significant association with the risk of HTN (p <0.0001). Other possible interactions are shown in **Figure 8.1.**

Table 8.4. Interaction models between genetic and sociodemographic factors.

Variable	CV training score	Testing score	CVC	*p-values*
Age	0.6574	0.6574	10/10	<0.0001
Age, rs1801133	0.678	0.6648	9/10	0.054
Age, rs1801133, Gender	0.6973	0.6593	7/10	<0.0001

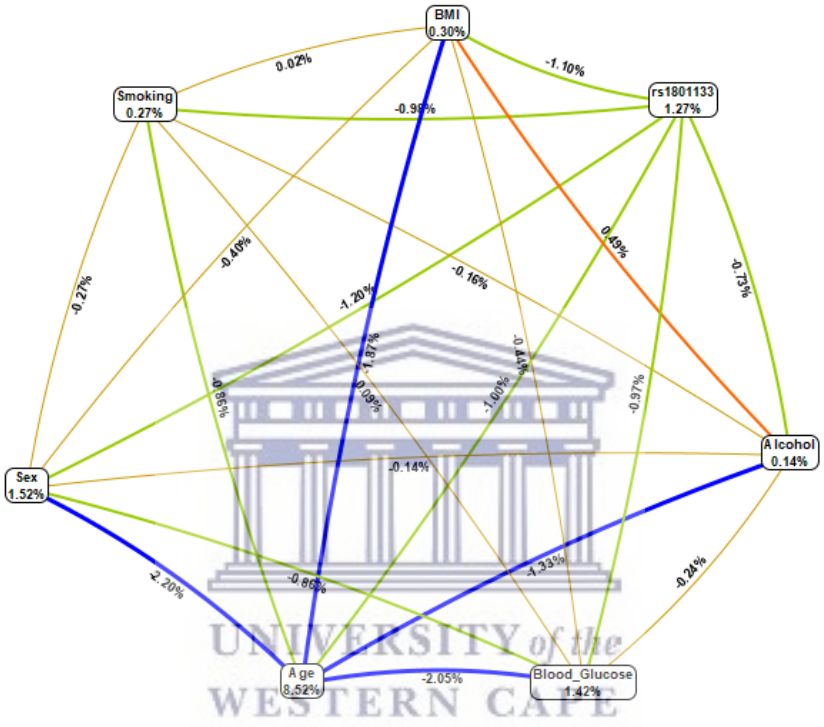

Figure 8.1 Multifactor dimensionality reduction combined attribute network demonstrating all possible interactions between rs1801133 and various environmental factors. Four different colours and various interactions between the genotypes of *MTHFR* (rs1801133) and selected variables (age, gender, smoking status, BMI, and blood glucose levels) were observed, and each colour represents a possible interaction. The width of the line indicates the strength of the interaction, whereas the thin lines represent weak interactions. Figures < 1 and thin lines represent weak interactions. The strongest interactions are represented by figures ≥1 and thick lines. The image was generated using the open-source MDR software package version 3.0.2.

8.4. Discussion

Previous data from our group demonstrated a high prevalence (~75%) of HTN in the villages of Mthatha, Eastern Cape [26, 27]. Through a narrative synthesis of the literature, we further reported that there is a lack of African-specific data with regards to the association between *MTHFR* rs1801133 and HTN [28, 29]; thus, highlighting the significance of the current report. The present study aimed to investigate the

association between *MTHFR* (rs1801133) and HTN in an indigenous South African population from the Eastern Cape province.

The current study reports on the lack of association between *MTHFR* (rs1801133) and the risk of HTN among an indigenous South African population (*n* = 442). Our findings are consistent with results reported by Amin *et al*, [23] and Amrani-Midoun *et al*. [9], where they also observed no association between *MTHFR* (rs1801133) and the risk of developing HTN in a cohort of Egyptian (*n* = 181) and Algerian (*n* = 154) adults, respectively. In contrast, a study done by Ghogomu *et al*. [8] showed a significant association between rs1801133 and HTN among a native Bantu ethnic group of the South-West region of Cameroon (*n* = 91). Furthermore, Nasseredine *et al*. [35] demonstrated that individuals of Moroccan (*n* = 203) origin who were homozygous carriers of rs1801133 were more likely to develop HTN.

Several studies performed in non-African countries also demonstrated that genotype TT or T allele of rs1801133 may increase the risk of HTN [6, 14, 15]. The discrepancies observed between these studies may be due to geographical location, ethnicity, and epigenetic mechanisms that are involved in the regulation of *MTHFR* gene expression [36, 37]. Suggesting that data linking HTN to rs1801133 remains controversial. Li *et al*. [38] and Rosenberg *et al*. [39] showed that the frequency of the T allele varies across different populations. The authors further demonstrated that individuals of African origin presented a low frequency of the T allele (< 10%) in comparison to other ethnic groups [40-42]. This was further confirmed by a study conducted by Atadzhanov *et al*. [43], who also observed a low frequency of the T allele in a Zambian population. In agreement with the later studies [43], our study demonstrated that the T allele and the TT genotype frequencies of *MTHFR* (rs1801133) were lower (5%) than the C allele (95%) in both hypertensive and non-hypertensive individuals. We, therefore, tested for Hardy-Weinberg equilibrium, and none of the genetic models deviated from the principle. This is an indication that the genotype and allele frequencies in our study population remained constant between generations, and that there was no random mating that occurred. Our findings further suggest that a positive association between *MTHFR* (rs1801133) polymorphism and HTN among African individuals may not be strong enough to withstand statistical interrogation by false-positive report probability [37]. Of note, the current study as well as the reference studies [8, 9, 23, 35], were composed of relatively small sample sizes; thus, the observations made remain to be explored in future studies with larger sample sizes.

Some observational studies have shown that genetic changes are inseparable from the impact of environmental factors such as diet, exercise, smoking, and drinking [44, 45]. As a result, SNP-environment interaction could not be ignored as our existing understanding of the pathogenesis of HTN indicates an interplay between genetic and environmental factors [6]. Although no association was established between rs1801133 and HTN in the present study, an interaction between rs1801133, age, and gender was observed,

suggesting a possible synergistic effect. This was in agreement with the previous studies which also reported that rs1801133, aging, and gender are potential risk factors for elevated Hcy levels [46], decreased uric acid [47], and folate deficiency [48], which increases the risk of HTN and hyperhomocysteinemia [49, 50]. Furthermore, Boers et al, [51] and Ueland et al, [52] have demonstrated that premenopausal women have lower Hcy levels than men and postmenopausal women [49, 50]. The reasons for the higher Hcy concentrations at older ages are not well understood, although changes in renal function are certainly involved. Higher Hcy concentrations in men in comparison to women may be explained by differences in muscle mass, hormone, and vitamin status [53]. Thus, this suggests that the interaction between age, gender, and the *MTHFR* (rs1801133) polymorphism in HTN may be partially due to their interactive effects on Hcy concentrations and DNA methylation status [6, 54-57]. But this hypothesis needs to be further explored in a bigger cohort.

8.4.1 Limitations and Strengths of this Study

It is noteworthy to mention that several limitations need to be considered in interpreting the findings of our study. Firstly, the sample size of our study was relatively small, of which could very likely introduce random errors and bias, resulting in unreliable results. However, this limitation projects another strength as it is often better to test a new research hypothesis on a small number of participants before progressing to a large number. This is especially important not to conserve resources. Importantly, our findings will be a stepping-stone to larger ongoing clinical and genetic studies of HTN in the South African population. Notably, also highlights another strength of the current report, as very few studies have addressed this research niche in a purely black African population. Importantly, unlike other reports [8, 35], our study did perform an adjustment for confounding factors such as gender, age, and smoking status. This indicates an urgent need to carefully plan African-specific studies with large sample sizes to be able to draw conclusions on the association between *MTHFR* (rs1801133) and HTN. Secondly, this study used a cross-sectional design, which precludes causal inference, consistent with recruiting participants from only one ethnic group (Xhosa population of Mthatha, Eastern Cape). Further highlights that our findings are generalised to the included study population and individuals residing in similar settings in the province. Lastly, the current project was planned to recruit approximately 430 participants, with a 1:1 ratio of cases to controls. But, due to the high prevalence of the HTN (~75%) in our study population, a high number of hypertensive patients were included in the study (n = 279). However, the T allele frequency of study population was not influenced by this limitation as it remained low (~5%). Despite these limitations, to the best of our knowledge, this is the first study to investigating the association between the *MTHFR* (rs1801133) variant and HTN in South Africa, and further explored a unique and important feature of SNP-environment interaction. Overall, these findings remain important in addressing the limited data on the *MTHFR* variant (rs1801133) and the risk of developing HTN in indigenous populations of South Africa.

8.5. Conclusions

In the present study, we demonstrated the lack of association between *MTHFR* (rs1801133) and HTN among individuals of Xhosa origin. However, a potential synergistic effect of the rs1801133 with age and gender on HTN susceptibility was observed. The current study has laid a foundation for future studies by highlighting the significance of considering the impact of environmental factors in genetic association studies. Furthermore, future studies with a large sample size are required to further validate our findings.

8.6. Authors Contributors

S.E.M and R.J conceptualised and designed the study. S.E.M acquired the data. S.E.M, C.M. J.R.S. and S.S, analysed and interpreted the data and drafted the manuscript. B.M., S.N, S.E.M, S.S, T.A, L.M, J.R.S, M.B. H.F. and R.J. were involved in the critical revision of the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

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8.9. Conflicts of Interest

The authors declare there are no conflicts of interest.

8.10. References

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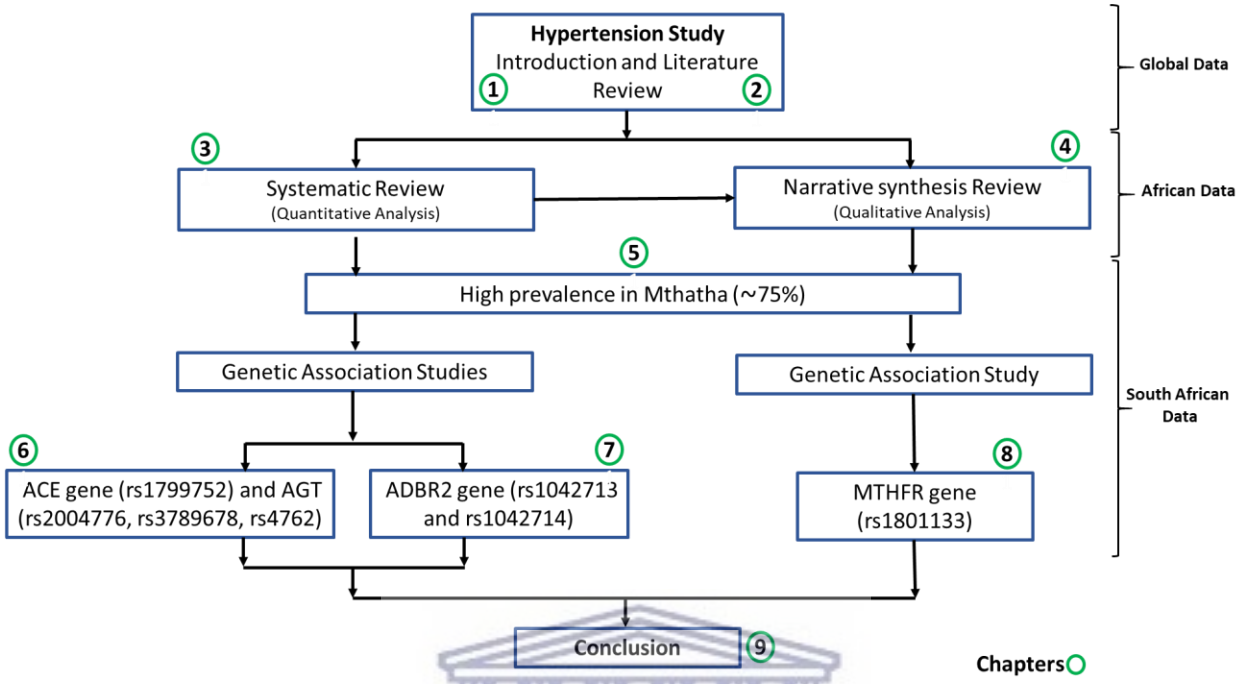
CHAPTER NINE

Graphic Abstract



9.1 Conclusion and Future Work

Hypertension (HTN) is a serious global health concern associated with the development of adverse health outcomes and poor quality of life. Despite having an advanced understanding of the pathogenesis of HTN and the availability of multiple antihypertensive medications, there is still a consistent rise in the prevalence of HTN which is a serious public health issue. Strong empirical evidence supports and confirms, that HTN is caused by a complex interplay of genetic, epigenetic, and various lifestyle factors. Genetic factors are been suggested to have a significant contribution to blood pressure variation, however, genetic factors could only explain > 3% of BP variance, meaning that many genetic variations are still needed to be explored [1]. As such, numerous studies have been conducted to investigate the potential of genetic factors in the risk of developing HTN. However, the findings remain inconsistent among different ethnic groups. Thus, the present study sought to investigate the inter-individual genetic variations and the development of HTN in an indigenous South African population from Mthatha, Eastern Cape. province (South Africa), and further compare the obtained data to the clinical outcome of HTN.

9.2 Summary of the results

The present study has achieved some important scientific observations. **Firstly**, we systematically extracted and discussed African evidence on genetic variation and pharmacogenomics toward the treatment of

HTN **(chapter 3)**. The systematic review was performed using publicly available databases searching for relevant papers between 1984 and 2020 following the HuGENetTM in gene association studies. A total of 2784 articles were identified and reviewed, but only 42 studies met the inclusion criteria. The selected 42 studies investigated a total of 53 genes in association with HTN and, only 20 studies reported a significant association with HTN development within the African population [2]. Among those, candidate genes such as *ACE, AGT, NOS3, ATP2B1,* and *MTHFR* have repeatedly been implicated to be associated with HTN in various African populations. In our systematic review, we have confirmed the scarcity of genetic data in the African continent in comparison with the available global data. Furthermore, a gene-drug interaction and ontology analysis were conducted to understand drug interaction with the shortlisted genes. Out of the 53 included genes, only 14 showed interactions with FDA-approved HTN drugs. Also, we further used *in-silico* methods to identify potential mechanisms that can be targeted for the identification of new novel drug targets. Furthermore, we identified three clusters from the 53 prioritized genes in association with HTN. Upon further analysis, genes such as *AGTR1, AGTR2, AGT,* and *ACE* (Cluster 1); *IGFBP2* (Cluster 2*),* and *ADRB2* (cluster 3) were shown to be mapped to FDA-approved HTN drugs. Interestingly, the protein-coding genes including *AGTR1, AGTR2, AGT,* and *ACE* are all involved in the RAAS cascade which regulates blood pressure and vascular resistance [3]. The latter is of importance, as the targeting of co-regulatory gene clusters would aid in the development of effective African-based individualised antihypertensive medicine. Henceforth, the opacity of the evidence emphasizes the need for larger genetic association studies such as HTN genetic studies to identify reliable and valid SNPs that are linked with HTN.

Following this *in silico* evaluation, a narrative synthesis review was performed for SNP (rs1801133) located in the *MTHFR* gene, where we have gathered and analysed available genetic evidence on *MTHFR* (rs1801133) and its association with the risk of HTN in the African population, and further compared the evidence with the available global data as discussed in chapter 4 [4]. Our narrative synthesis review identified 64 potential studies globally, of which 4 studies were from the African continent, which highlights the scarcity of genetic studies on the association between rs1801133 SNP and HTN in Africa, especially in the black African population. Furthermore, our study reported that there is no association between *MTHFR* locus and risk of developing HTN. However, the result from the current review opened avenues to further explore a possible association between rs1801133 and HTN among individuals of African origin. Thus, our recommendation is that future black African population studies with a large sample size are required to further confirm these findings and explain the *MTHFR* pathogenesis of HTN in an African population.

Next, this study determined the prevalence and associated risk factors of HTN in the indigenous South African population of Mthatha Town (n = 556) (Eastern Cape) (**chapter 5**). The results obtained suggested that the prevalence of HTN was high (~71%) in the indigenous South African population of Mthatha town, Further, our results revealed a positive association between age, income, westernized diet, blood glucose levels, and BMI, and HTN. Additionally, it has also been demonstrated that gender, age, education, BMI, and belief in controlling HTN with medication are key predictors of determining the treatment status of HTN. This study observed that about half of the participants were unaware of their HTN status which highlights the urgent need for HTN education, screening, and control within the Mthatha area. This study showed that approximately 40% of participants were unaware of their HTN status and as such highlights the urgent need for HTN education, screening, and control within the Mthatha area. Fourthly, Mabhida *et al*, [2] reported on 3 possible clusters that might play a role in HTN progression, with genes in cluster one being linked to an overactive RAS pathway, with RAS playing a critical role in BP homeostasis. Hence, SNP within genes linked to the RAS pathway was explored in **chapter 3** (figure 3.5). First and foremost, this chapter reported that *AGT*, *AGTR1*, *AGTR2*, and *ACE* were co-expressed (cluster 1), and as such, could possibly be identified as potential multiple drug targets. These gene polymorphisms in the RAS pathway may play a role in predicting vulnerability to HTN. Therefore, this study sought to investigate the role of identified *ACE* and *AGT* polymorphisms in predicting HTN in our study population (n = 177) **(chapter 6)**. The results obtained showed no significant association for both *ACE* and *AGT* polymorphisms in the univariate analysis, however, a multivariate analysis showed that the T allele provides a 3-fold risk of developing HTN for rs2004776, while rs3789678 offers protection. Furthermore, haplotype analysis revealed rs2004776 and rs3789678 to be in strong linkage disequilibrium. These findings demonstrated that rs2004776, rs3789678, and rs4762 located within the *AGT* gene might be a strong predictor of HTN. As such, future studies will include a follow-up study where a larger sample size will be obtained to confirm this association.

This study also investigated the association between two common beta 2-adrenergic receptor gene (*ADRB2*, Cluster 3*)* polymorphisms (rs1042713 and rs1042714), and the risk of HTN in the indigenous South African population of the Mthatha Town, Eastern Cape (n = 442) **(chapter 7).** Beta 2-adrenergic receptor gene (*ADRB2*) has long been implicated in the sympathetic nervous system and the regulation of blood pressure. Although several *ADBR2* SNPs are associated with HTN, the evidence for *ADRB2* gene association with HTN remains enigmatic. Our results showed no significant association between both rs1042714 and rs1042713 polymorphisms and the risk of developing HTN in our population. However, a potential synergistic effect occurred among rs1042713 genotypes, BMI, and age on HTN in our study population. However, future studies with a large sample size are required to further validate these findings and study the role of these *ADRB2* polymorphisms in the risk of developing HTN.

Lastly, in **chapter 4**, this study made use of a narrative synthesis review to show that there is a lack of African-specific data regarding the association between *MTHFR* rs1801133 and HTN. Thus, based on these results, a follow-up short communication study was performed (**chapter 8**), where we investigated the association between the *MTHFR* (rs1801133) variant and the risk of developing HTN in the Mthatha population (n = 442). In agreement with the study by Amin *et al*, [16], our study found no significant association between *MTHFR* (rs1801133) and the risk of developing HTN in an indigenous South African population. The results from our study suggest that rs1801133 polymorphism might not be a risk factor for developing HTN in our population, however further studies are required to confirm these findings. Although, our findings showed no association between the *MTHFR* (rs1801133) and HTN, interaction among the rs1801133, age, and gender was established, suggesting a possible synergistic effect of the rs1801133 with age and sex on HTN susceptibility. These results suggest that the interaction between age and the *MTHFR* (rs1801133) polymorphism on HTN may be partially due to their interactive effects on homocysteine concentrations and DNA methylation status, although further studies are required to explore this hypothesis.

9.3 Limitations of This Study

It is noteworthy to mention that in interpreting the findings of our study, several limitations need to be considered. (1) the sample size of our study was relatively small. In molecular epidemiological studies, small-sample size research is very likely to have random errors and bias, resulting in unreliable results [5]. Therefore, our results should be interpreted with caution until more studies on these underrepresented groups accumulate. However, this will be a stepping-stone for larger ongoing clinical and genetic studies of HTN in the South African population (2) Men were underrepresented in the studied population which prevented us from a complete understanding of the gender-based contribution to the prevalence of HTN. (3) We could not do an in-depth assessment of dietary habits and physical activity patterns; therefore, findings should be explicated with caution. (4), this study used a cross-sectional design, which precludes causal inference, consistent with recruiting participants from only one ethnic group (Xhosa population of Mthatha, Eastern Cape). Further highlights that our findings are generalised to the included studied population and individuals residing in similar settings in the province. (5), Due to the questionnaire used to collect personal exposure information (such as smoking and drinking), the responses might be influenced by the participant's memory, so recall bias cannot be excluded.

9.4 Strengths of This Study

Despite these limitations, to the best of our knowledge, this is the first study to investigate the association of the different genetic variants such as rs2004776, rs3789678, and rs1042713 with the HTN development in the indigenous South African population of Mthatha Town, Eastern Cape and further explores a unique

and important feature of SNP-environment interaction, which opens the door for future research. Furthermore, our findings have highlighted the scarcity of genetic information concerning HTN in South Africa. While the sample size of the present study was relatively small, this limitation projects another strength as it is often better to test a new research hypothesis on a small number of participants first. This helps to avoid spending too many resources such as time and financial costs, on finding an association between risk factors and the disease when there really is no association [6]. Notably, also highlights another strength of the current report, as very few studies have addressed this research niche in indigenous South African populations. This indicates an urgent need for future genetic studies including the indigenous South African population with large sample size. Overall, these findings address the critical issue of having limited data available on the association of SNPs with the risk of developing HTN in the indigenous population of South Africa.

9.5 Novelty and significance of the study

HTN is now becoming a serious South African public health issue associated with the poor quality of life. Taken together, the experimental results from this study have demonstrated that the prevalence of HTN was high among the black rural African population of Mthatha Town. It is also revealed that age, income, westernized, higher levels of blood glucose and BMI are positively associated with HTN. These findings also highlight the changing patterns of dietary habits among the rural communities of South Africa. Furthermore, this study has demonstrated that *AGT* (rs2004776, rs3789678) polymorphisms may offer potential as predictors of HTN in the black African population and may be used in precision medicine in future research studies.

To the best of our knowledge, this is the first study to evaluate the association between various genetic polymorphisms such as rs2004776, rs1042713 and rs3789678 and the risk of developing HTN in the South African population from Mthatha. While the allele frequency of genetic polymorphisms such as *MTHFR* (rs1801133), *ADRB2* (rs1042714), and *AGT* (rs4762) showed to be too low to detect the risk of HTN in our study population, genetic polymorphisms of these variants were found to be associated with HTN in other countries outside Africa. Upon further analysis, genes such as *AGTR1*, *AGTR2*, *AGT*, and *ACE* (Cluster 1); *IGFBP2* (Cluster 2), and *ADRB2* (cluster 3) were shown to be mapped to FDA-approved HTN drugs. Interestingly, the protein-coding genes including *AGTR1*, *AGTR2*, *AGT*, and *ACE* are all involved in the RAAS cascade which regulates blood pressure and vascular resistance. The latter is of importance, as the targeting of co-regulatory gene clusters would aid in the development of effective African-based individualised antihypertensive medicine. Hereafter, the opacity of the evidence emphasizes the need for larger genetic association studies such as HTN genetic studies to identify reliable and valid SNPs that are linked with HTN.

9.6 Implications for future research

The findings of this study laid the basis for future research. Firstly, to perform the post *in silico* mass-array genotyping analysis of genetic variants (rs2004776, rs3789678) that were found to be associated with HTN. Secondly, to show the disease mechanism in relation between genetic variants (rs2004776, rs3789678) and HTN, and to investigate the effect of these SNPs on the gene architecture. Lastly, to evaluate and identify genetic variants in hypertensive patients with concomitant T2DM in the Xhosa South African population.

9.7 References

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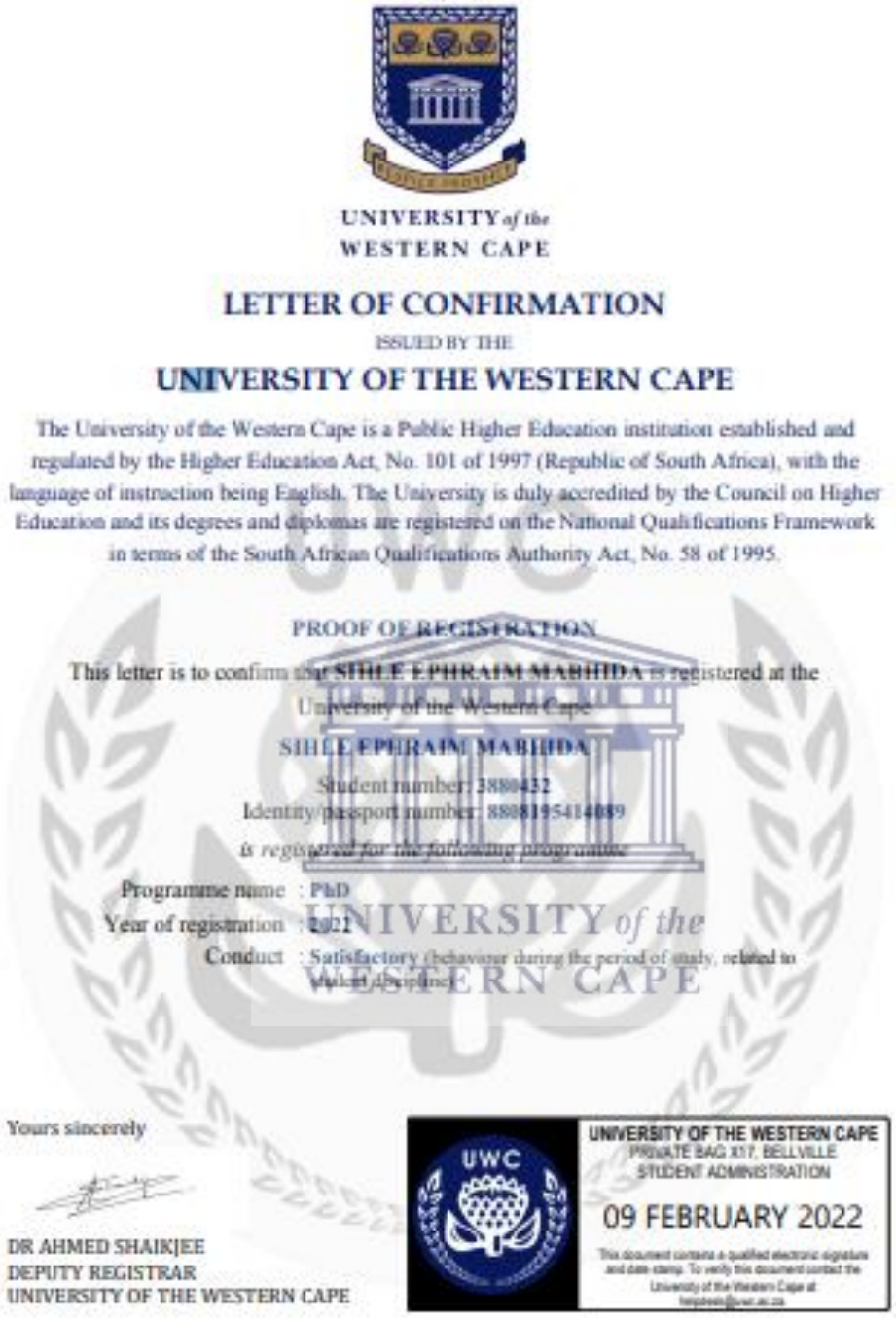
Appendix 1: Proof of registration



UNIVERSITY *of the*
WESTERN CAPE

LETTER OF CONFIRMATION

ISSUED BY THE

UNIVERSITY OF THE WESTERN CAPE

The University of the Western Cape is a Public Higher Education institution established and regulated by the Higher Education Act, No. 101 of 1997 (Republic of South Africa), with the language of instruction being English. The University is duly accredited by the Council on Higher Education and its degrees and diplomas are registered on the National Qualifications Framework in terms of the South African Qualifications Authority Act, No. 58 of 1995.

PROOF OF REGISTRATION

This letter is to confirm that **SIHLE EPHRAIM MABHIDA** is registered at the University of the Western Cape

SIHLE EPHRAIM MABHIDA

Student number: 3880432
Identity/passport number: 8808195414089
is registered for the following programme

Programme name : PhD
Year of registration : 2021
Conduct : Satisfactory (behaviour during the period of study, related to student discipline)

Yours sincerely

DR AHMED SHAIKJEE
DEPUTY REGISTRAR
UNIVERSITY OF THE WESTERN CAPE

UNIVERSITY OF THE WESTERN CAPE
PRIVATE BAG X17, BELLVILLE
STUDENT ADMINISTRATION
09 FEBRUARY 2022

Appendix 2: Ethical Clearance from university of Western Cape

UNIVERSITY of the WESTERN CAPE

OFFICE OF THE DIRECTOR: RESEARCH
RESEARCH AND INNOVATION DIVISION

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South Africa
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13 November 2019

Prof M Benjeddou
Biotechnology
Faculty of Natural Sciences

Ethics Reference Number: BM19/8/19

Project Title: Inter-individual genetic variation and the development of uncontrolled hypertension in patients with concomitant type 2 diabetes mellitus in Eastern Cape, South Africa.

Approval Period: 08 November 2019 – 08 November 2020

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above mentioned research project.

Any amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report in good time for annual renewal.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer

Appendix 3: Ethical Clearance from the South African Medical Research Council

saMRC advancinglife

HUMAN RESEARCH ETHICS COMMITTEE

16 November 2020

Dr Rabia Johnson
BRIP
SAMRC Cape Town

Dear Dr Johnson

Protocol ID: EC028-8/2020
Protocol title: Elucidating inter-individual genetic variation in the development of uncontrolled hypertension – the role of individualised drug therapy for a sub-Saharan African population
Meeting date: 27 October 2020

Thank you for your application to the Committee, which was discussed at August 2020 meeting, and your responses dated 13 October and 11 November 2020. I am pleased to inform you that ethics approval is now granted for the study.

Please note that the approval is valid for 1 year, i.e. from 27 October 2020 to 26 October 2021. Any changes to the research protocol must be submitted as an amendment. Any serious adverse events must be reported within 48 hours. Any protocol deviations have to be reported.

Yours sincerely

Prof Danie du Toit
Chairperson: SAMRC Human Research Ethics Committee

Members at the May meeting: Prof D du Toit (Chairperson), Ms S Behardien, Adv J Early, Dr H Etheredge, Prof A Kengne, Ms M Ledwaba, Prof C Lombard, Dr A Loxton, Mr G Makanda, Dr E Nicol, Prof C Wiysonge, Dr W Zembe

Appendix 4: Ethical Clearance from the province of estern cape health

Province of the
EASTERN CAPE
HEALTH

Enquiries: Zonwabele Mertle
Email: zonwabele.mertle@echealth.gov.za
Tel no: 083 378 0202
Fax no: 043 642 1909

Date: 17 September 2020

RE: The management and building bridges for selected non-communicable disease and HIV Risk factors, morbidity and mortality in the EC: A population-, laboratory and community study. (EC_2017RP56_979)

Dear Dr S.A. Mabunda and Mr S.C. Nomatshila

The department would like to inform you that your application for extension of the abovementioned research has been approved based on the following conditions:

1. During your study, you will follow the submitted protocol with ethical approval and can only deviate from it after having a written approval from the Department of Health in writing.

2. You are advised to ensure, observe and respect the rights and culture of your research participants and maintain confidentiality of their identities and shall remove or not collect any information which can be used to link the participants.

3. The Department of Health expects you to provide a progress update on your study every 3 months (from date you received this letter) in writing.

4. At the end of your study, you will be expected to send a full written report with your findings and implementable recommendations to the Eastern Cape Health Research Committee secretariat. You may also be invited to the department to come and present your research findings with your implementable recommendations.

5. Your results on the Eastern Cape will not be presented anywhere unless you have shared them with the Department of Health as indicated above.

Your compliance in this regard will be highly appreciated.

SECRETARIAT: EASTERN CAPE HEALTH RESEARCH COMMITTEE

TOGETHER, MOVING THE HEALTH SYSTEM FORWARD

Appendix 5: Published Articles

1.

genes

MDPI

Review

Methylenetetrahydrofolate Reductase Polymorphism (rs1801133) and the Risk of Hypertension among African Populations: A Narrative Synthesis of Literature

Sihle E. Mabhida [1,2], Babu Muhamed [3], Jyoti R. Sharma [1], Teke Apalata [4,5], Sibusiso Nomatshila [6], Lawrence Mabasa [7], Mongi Benjeddou [2], Charity Masilela [7], Khanyisani Ziqubu [7], Samukelisiwe Shabalala [1,8,9] and Rabia Johnson [1,8,*]

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Abstract: In this review, we have gathered and analyzed the available genetic evidence on the association between the methylenetetrahydrofolate reductase gene (*MTHFR*), rs1801133 and the risk of Hypertension (HTN) in African populations, which was further compared to the global data evidence. This review was reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) protocol and Human Genome Epidemiology Network (HuGENet) guidelines. Literature was retrieved through major search databases, including PubMed, Scopus, Web of Science, and African Journal Online. We identified 64 potential studies, of which 4 studies were from the African continent and 60 studies were reported globally. Among the studies conducted in Africa, only two ($n = 2$) reported a significant association between the *MTHFR* (rs1801133) and the risk of developing HTN. Only one ($n = 1$) study population was purely composed of black Africans, while others were of other ethnicities. Among studies conducted in other continents ($n = 60$), forty-seven ($n = 47$) studies reported a positive association between *MTHFR* (rs1801133) and the risk of developing HTN, whereas the remaining studies ($n = 14$) did not show a significant association. Available literature suggests an apparent association between rs1801133 and HTN in global regions; however, such information is still scarce in Africa, especially in the black African population.

Keywords: Hypertension; methylenetetrahydrofolate reductase gene; *MTHFR*; single-nucleotide polymorphism; Africa; genetic variation

1. Introduction

Hypertension (HTN) remains a major risk factor for the development of cardiovascular diseases (CVDs), which significantly contributes to high rates of mortality and morbidity worldwide. Globally, HTN affects over 1.4 billion individuals above the age of 18 years and the number is expected to increase to 1.56 billion by 2025 [1–3]. In Africa, HTN

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Hypertension in African Populations: Review and Computational Insights

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Abstract: Hypertension (HTN) is a persistent public health problem affecting approximately 1.3 billion individuals globally. Treatment-resistant hypertension (TRH) is defined as high blood pressure (BP) in a hypertensive patient that remains above goal despite use of ≥3 antihypertensive agents of different classes including a diuretic. Despite a plethora of treatment options available, only 31.0% of individuals have their HTN controlled. Interindividual genetic variability to drug response might explain this disappointing outcome because of genetic polymorphisms. Additionally, the poor knowledge of pathophysiological mechanisms underlying hypertensive disease and the long-term interaction of antihypertensive drugs with blood pressure control mechanisms further aggravates the problem. Furthermore, in Africa, there is a paucity of pharmacogenomic data on the treatment of resistant hypertension. Therefore, identification of genetic signals having the potential to predict the response of a drug for a given individual in an African population has been the subject of intensive investigation. In this review, we aim to systematically extract and discuss African evidence on the genetic variation, and pharmacogenomics towards the treatment of HTN. Furthermore, in silico methods are utilized to elucidate biological processes that will aid in identifying novel drug targets for the treatment of resistant hypertension in an African population. To provide an expanded view of genetic variants associated with the development of HTN, this study was performed using publicly available databases such as PubMed, Scopus, Web of Science, African Journal Online, PharmGKB searching for relevant papers between 1984 and 2020. A total of 2784 articles were reviewed, and only 42 studies were included following the inclusion criteria. Twenty studies reported associations with HTN and genes such as *AGT* (rs699), *ACE* (rs1799752), *NOS3* (rs1799983), *MTHFR* (rs1801133), *AGTR1* (rs5186), while twenty-two studies did not show any association within the African population. Thereafter, an in silico predictive approach was utilized to identify several genes including *CLCNKB*, *CYP11B2*, *SH2B2*, *STK39*, and *TBX5* which may act as potential drug targets because they are involved in pathways known to influence blood pressure. Next, co-expressed genes were identified as they are controlled by the same transcriptional regulatory program and may potentially be more effective as multiple drug targets in the treatment regimens for HTN. Genes belonging to the co-expressed gene cluster, *ACE*, *AGT*, *AGTR1*, *AGTR2*, and *NOS3* as well as *CSK* and *ADRG1* showed enrichment of G-protein-coupled receptor activity, the classical targets of drug discovery, which mediate cellular signaling processes. The latter is of importance, as the targeting of co-regulatory

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Prevalence of Hypertension and Its Associated Risk Factors in a Rural Black Population of Mthatha Town, South Africa

Jyoti Rajan Sharma [1], Sihle E. Mabhida [1,2], Bronwyn Myers [3,4], Teke Apalata [5], Edward Nicol [6,7], Mongi Benjeddou [2], Christo Muller [1,8] and Rabia Johnson [1,8,*]

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Abstract: *Background:* The occurrence of hypertension has been increasing alarmingly in both low and middle-income countries. Despite acknowledging hypertension as the most common life-threatening risk factor for cardiovascular disease (CVD), a dearth of data is available on the prevalence, awareness, and determinants of hypertension in rural parts of South Africa. The principal aim of the current study is to determine the prevalence and associated risk factors of hypertension among a black rural African population from the Mthatha town of Eastern Cape Province. *Methods:* This was a cross-sectional study, and individuals over 18 years of age were randomly screened using a World Health Organization stepwise questionnaire. Sociodemographic information, anthropometric measurements, fasting blood glucose levels, and three independent blood pressure (BP) readings were measured. Blood pressure measurements were classified according to the American Heart Association guidelines. Univariate and multivariate analyses were performed to determine the significant predictors of hypertension. *Results:* Of the total participants (n = 556), 71% of individuals had BP scores in the hypertensive range. In univariate analysis, age, westernized diet, education, income, and diabetic status, as well as overweight/obese status were positively associated with the prevalence of hypertension. However, in a multivariate logistic regression analysis only, age, body mass index (BMI), diabetic status, and westernized diet were significantly associated with a higher risk of developing hypertension. Gender, age, and BMI were potential factors having a significant association with the treatment of hypertension. Individuals who did not consider the importance of medicine had higher chances of having their hypertension being untreated. *Conclusions:* Prevalence of hypertension was high among the black rural African population of Mthatha town. Gender, age, westernized diet, education level, income status, diabetic as well as overweight/obese status were the most significant predictors of hypertension.

Keywords: hypertension prevalence; body mass index; factors; hypertension treatment

4.

Pharmacogenomics of amlodipine and hydrochlorothiazide therapy and the quest for improved control of hypertension: a mini review

Rabia Johnson[1,2] · Phiwayinkosi Dludla[1] · Sihle Mabhida[1,3] · Mongi Benjeddou[3] · Johan Louw[1] · Faghri February[4]

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Abstract
Blood pressure (BP) is a complex trait that is regulated by multiple physiological pathways and include but is not limited to extracellular fluid volume homeostasis, cardiac contractility, and vascular tone through renal, neural, or endocrine systems. Uncontrolled hypertension (HTN) has been associated with an increased mortality risk. Therefore, understanding the genetics that underpins and influence BP regulation will have a major impact on public health. Moreover, uncontrolled HTN has been linked to inter-individual variation in the drugs' response and this has been associated with an individual's genetics architecture. However, the identification of candidate genes that underpin the genetic basis of HTN remains a major challenge. To date, few variants associated with inter-individual BP regulation have been identified and replicated. Research in this field has accelerated over the past 5 years as a direct result of on-going genome-wide association studies (GWAS) and the progress in the identification of rare gene variants and mutations, epigenetic markers, and the regulatory pathways involved in the pathophysiology of BP. In this review we describe and enhance our current understanding of how genetic variants account for the observed variability in BP response in patients on first-line antihypertensive drugs, amlodipine and hydrochlorothiazide.

Keywords Amlodipine · Blood pressure, hydrochlorothiazide · Hypertension · Single nucleotide polymorphisms (SNPs)

Introduction

Hypertension (HTN) often called the "silent killer" is defined as a recurrent systolic/diastolic Blood pressure (BP) higher than 140/90 mmHg. It affects approximately 1.13 billion people with an estimated 7.5 million deaths globally [1]. Of great concern is the 30–40% of working age adults (25–64 years of age) that are hypertensive, and of which only one third have their BP under control [1, 2]. Uncontrolled BP is associated with increased risk for stroke and coronary artery disease, which are the leading causes of death worldwide. Hypertension has not only become a public health problem but a key national and international priority as uncontrolled HTN imposes a heavy cost on a country's national budget, health care services, and individual households. Similarly, in Sub-Saharan Africa, HTN is classified as the number one mortality risk with an estimated 500,000 deaths, affecting approximately 10 million South Africans with an associated 60–75% uncontrolled cases [3, 4]. Furthermore, only 27% of HTN patients are aware of their status, and only 18% are on

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5.

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The association of *MTHFR* (rs1801133) with hypertension in an indigenous south African population

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Aims: The current study sought to investigate the association between the methylenetetrahydrofolate reductase (*MTHFR*) variant (rs1801133) and the risk of developing hypertension (HTN) in an indigenous South African population.

Methods: A total of 442 participants (hypertensive, *n* = 279 and non-hypertensive, *n* = 163) from the indigenous tribe residing in Mthatha, Eastern Cape (South Africa) were recruited. HTN was defined as a systolic (SBP) and diastolic blood pressure (DBP) of ≥140/90 mmHg following American Heart Association guidelines. The genotyping of *MTHFR* (rs1801133) was assessed using KASPar SNP system. Thereafter, the association between rs1801133 in various genetic models and HTN was determined by logistic regression model analysis. Furthermore, the interaction between rs1801133 and selected risk factors on HTN was performed using the open-source multifactor dimensionality reduction (MDR).

Results: The low frequency of the T allele (5%) was also observed when compared with the C allele (95%) in both cases and controls. After adjusting for confounding factors (gender, smoking status, BMI, and blood glucose levels), there were no significant associations were observed between rs1801133 and the risk of HTN in all genetic models: genotypic (OR 0.75, 95% CI 0.29–1.95, *p* = 0.56), dominant (OR 0.86, 95% CI 0.35–2.16, *p* = 0.75), co-dominant (OR 1.33, 95% CI 0.51–3.48, *p* = 0.55) and allelic (OR 0.80, 95% CI 0.49–1.62, *p* = 0.70) in logistic regression analysis. However, a significant interaction was reported among rs1801133, age, and gender (*p* < 0.0001) with the risk of HTN.

Appendix 6: Turnitin report

SE MABHIDA

ORIGINALITY REPORT

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5	Shao-Liang Zhu, Yun Zhao, Xue-Ying Hu, Tao Luo, Zu-Shun Chen, Yu Zhang, Shui-Hua Yang, Lu Zhou, Le-Qun Li. "Genetic polymorphisms -137 (rs187238) and -607 (rs1946518) in the interleukin-18 promoter may not be associated with development of hepatocellular carcinoma", Scientific Reports, 2016	2%