Investigation of Socio-demographic, Clinical and Genetic Factors Associated with Blood Pressure and Glycaemic Control among Indigenous South African Adult Patients

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Abstract

Background: Achieving blood pressure and glycaemic treatment targets remain a major public health challenge in individuals with hypertension and diabetes mellitus (DM). This research project was, therefore, designed to investigate the socio-demographic, clinical and genetic factors associated with blood pressure and glycaemic control among indigenous South African adult patients. The main aims of the project were as follows:

(1) To assess the prevalence and socio-demographic factors associated with uncontrolled hypertension, in individuals receiving chronic care in primary healthcare facilities, based in the rural areas of Mkhondo Municipality (Study 1).

(2) To investigate the association of nineteen single nucleotide polymorphisms (SNPs) with blood pressure control among adult patients treated with hydrochlorothiazide (Study 2).

(3) To assess the level of association between twelve SNPs with uncontrolled blood pressure for adult patients treated with amlodipine (Study 3).

(4) To examine the association of five SNPs in selected genes (ABO, VEGFA, BDKRB2, NOS3 and ADRB2) with blood pressure response to enalapril treatment, and further assess interaction patterns that influence blood pressure response (Study 4).

(5) To determine the prevalence of poor glycaemic control and its influencing factors among adult patients from Mkhondo Municipality attending chronic care for DM (Study 5).

(6) To evaluate the level of association between polymorphisms found in the SLC22A1, SP1, PRPF31, NBEA, SCNN1B, CPA6 and CAPN10 genes, and glycaemic response to metformin and Sulphonylureas (SU) combination therapy among South African adults with DM. Also, to investigate interaction patterns that influence glycaemic control in response to metformin and SU combination therapy (Study 6).

ii

Methods: A total of 614 individuals attending chronic care for DM and hypertension were recruited at three primary health care centres in the rural Mkhondo Municipality of Mpumalanga province and a peri-urban regional hospital in Mdantsane Township in the Eastern Cape Province, South Africa. Uncontrolled hypertension was defined as systolic blood pressure ≥ 140mmHg and/or diastolic blood pressure ≥ 90mmHg in accordance with the South African Hypertension Society guideline (2014). Glycaemic control status was categorized as poor if glycated haemoglobin (HbA1c) > 7% and very poor if HbA1c ≥ 9%. SNPs previously associated with selected anti-hypertensive and anti-diabetic drugs were genotyped using MassArray. Multivariate regression analysis was used to identify the significant determinants of glycaemic and blood pressure responses.

Results: The results from this project are presented in six independent chapters (3 – 8) covering the performed studies.

Study 1: A total of 329 participants were hypertensive. The majority of the participants were 55 years old and above (69.0%), Zulus (81.2%), non-smokers (84.19%) and had been diagnosed with hypertension for more than a year prior to the study (72.64%). The overall prevalence of uncontrolled hypertension was 56.83% (n=187) with no significant difference between sexes, 57.38% males versus 56.88% females, respectively. In the multiple logistic regression model analysis after adjusting for confounding variables, obesity (AOR=2.90; 95% CI 1.66-5.05), physical activity (AOR=4.79; 95% CI 2.15-10.65) and HDL-C (AOR=5.66; 95% CI 3.33-9.60) were the significant and independent determinants of uncontrolled hypertension in the cohort.

Study 2: A total of 291 hypertensive individuals undergoing hydrochlorothiazide therapy were selected. About 73.19% and 54.98% of the study participants were female and Xhosa respectively. Majority of the cohort had blood pressure ≥140/90 mmHg (68.73%). Seventeen

SNPs were observed among the Xhosa tribe and two (rs2070744 and rs7297610) were detected among Swati and Zulu participants. Furthermore, Allele T of rs2107614 (AOR=6.69; 95%CI 1.42-31.55; p=0.016) and C of rs2776546 (AOR=3.78; 95%CI 1.04-13.74; p=0.043) were independently associated with uncontrolled hypertension. Whilst rs2070744 TC (AOR=38.76; 95%CI 5.54-270.76; p=0.00023), CC (AOR=10.44; 95%CI 2.16-50.29; p=0.003) and allele T of rs7297610 (AOR=1.86; 95%CI 1.09-3.14; p=0.023) were significantly associated with uncontrolled hypertension among Zulu and Swati participants.

Study 3: A total 304 hypertensive participants were on amlodipine therapy. The cohort was comprised of 76.64% females, 89.13% individuals who were older than 45 years and 52.31% of individuals with uncontrolled hypertension. Of the 12 SNPs genotyped, five SNPs; rs1042713 (MAF=45.9%), rs10494366 (MAF=35.3%), rs2239050 (MAF=28.7%), rs2246709 (MAF=51.6%) and rs4291 (MAF=34.4%) were detected among the Xhosa participants, while none were detected among the Swati and Zulu tribal groups. Variants, rs1042713 and rs10494366, demonstrated an expression frequency of 97.55% and 79.51%, respectively. Variant TA genotype of rs4291 was significantly associated with uncontrolled hypertension. No association was established between blood pressure response to amlodipine and the remaining four SNPs.

Study 4: The cohort was comprised of 284 participants on enalapril treatment, of which the majority were female (75.00%), Xhosa (78.87%) and had uncontrolled hypertension (69.37%). All five SNPs were exclusively expressed among Swati and Zulu participants. In the multivariate (adjusted) logistic model analysis, ADRB2 rs1042714 GC [AOR=2.35; 95%CI 1.09-5.05; p=0.029], BDKRB2 rs1799722 CT [AOR=2.20; 95% CI 1.03-4.72; p=0.041] and C allele [AOR=0.37; 955CI 0.15-0.94); p=0.037] were independently associated with

controlled hypertension in response to enalapril. A significant interaction between rs699947, rs495828 and rs2070744 (CVC= 10/10; p=0.0005) in response to enalapril was observed.

Study 5: The study was comprised of 157 individuals with DM. The majority of the study participants were females (84.71%) and above 45 years old (88.55%). The overall prevalence of poor glycaemic control was 77.71% (n=122), whilst extremely poor glycaemic control occurred in 50.6% (n=80) of the study cohort. In the multivariate logistic regression model analysis, African traditional (AOR=0.15; 95%CI 0.04-0.57), fast food consumption (AOR=5.89; 95%CI 2.09-16.81), elevated TC (OR=2.33; 95%CI 1.50-5.17), elevated LDL-C (AOR=5.28; 95%CI 1.89-14.69) and TG (AOR=4.39; 95%CI 1.48-13.00) were the independent and significant determinants of poor glycaemic control. Age (AOR=0.46; 95%CI 0.23-0.92) was the only independent and significant determinant of very poor glycaemic control.

Study 6: A total of 128 patients on metformin and sulfonylurea combination therapy were selected, of whom 85.93% (n=110) were female and 77.34% (n= 99) had uncontrolled T2DM (HbA1c >7%). In the multivariate (adjusted) logistic regression model analysis, the CC genotype of rs2162145 (*CPA6*), GG and GA genotypes of rs889299 (*SCNN1B*) were significantly associated with uncontrolled T2DM. On the other hand, the C allele of rs254271 (*PRPF31*) and the GA genotype of rs3792269 (CAPN10) were associated with controlled T2DM. A significant interaction between rs2162145 and rs889299 in response to metformin and SU combination therapy was observed.

Conclusions: The prevalence of uncontrolled hypertension and diabetes was investigated among indigenous South African adult patients, as well as their associated risk factors. All patients were receiving treatment in rural public clinics serving the communities of Mkhondo municipality in the Mpumalanga Province. The main socio-demographic and clinical factors,

v

which showed association with failing to achieve blood pressure and glycaemic targets, included unfavorable lifestyle and/or diets, as well as dyslipidemia. Measures aimed at changing dietary and lifestyle pattens should, therefore, be implemented even when taking drug treatment for hypertension and DM. In addition, panels of previously identified genetic biomarkers, and showing association with antihypertensive and antidiabetic drug responses, were investigated for possible use in treatment selection and optimization. Polymorphisms rs2107614, rs2776546, rs2070744 and rs7297610 were significantly associated with uncontrolled hypertension for patients treated with hydrochlorothiazide. In the case of enalapril, rs1042714 and rs1799722 were independently associated with controlled hypertension in response to treatment, while rs4291 was significantly associated with uncontrolled hypertension for amlodipine. On the other hand, rs2162145, rs889299, rs25427 and rs3792269 were associated with glycaemic response to metformin and SU combination therapy, including variant-specific improved or poor DM control. Further pharmacogenomics studies should be conducted to validate the investigated genetic biomarkers included in this study, and to evaluate additional genetic variants for possible use in optimising therapies for HPT and DM patients in terms of drug choice and dose.

Keywords

Amlodipine

Combination therapy

Diabetes Mellitus

Enalapril

Gene-Gene interaction

Hydrochlorothiazide

Hypertension

MassArray

MDR

Metformin

Non-communicable Diseases

Pharmacogenomics

Precision Medicine

Single Nucleotide Polymorphisms

Sulphonylureas

Uncontrolled Diabetes

Uncontrolled Hypertension

Declaration

I, **Charity Mandisa Masilela** declare that *'Investigation of Socio-demographic, Clinical and Genetic Factors Associated with Blood Pressure and Glycaemic Control Among indigenous South African Adult Patients'* 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.

Signature… … **Date…06/08/2021…………………**

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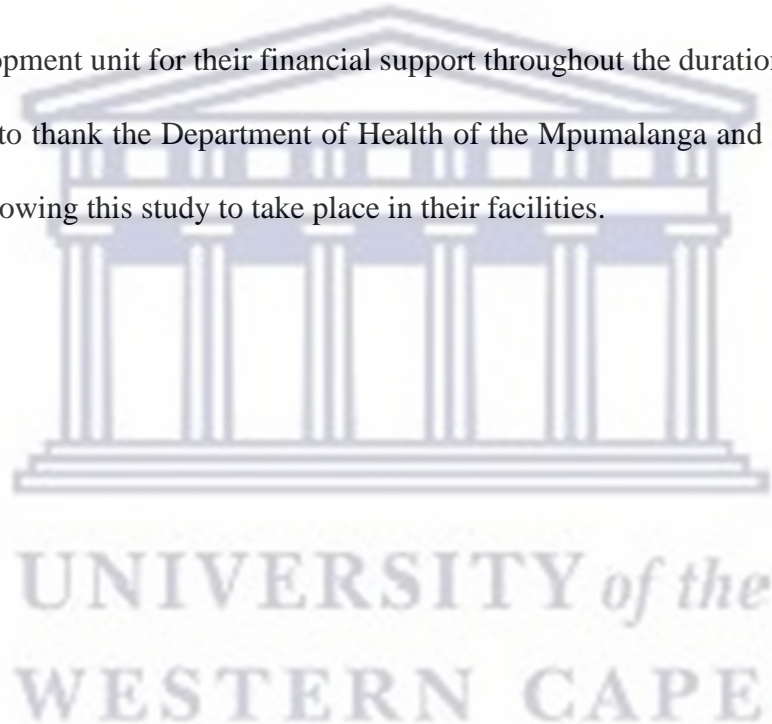
Dedication

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

Journal articles – Published

Masilela C, Pearce B, Ongole JJ, Adeniyi OV, Benjeddou M. Cross-sectional study of prevalence and determinants of uncontrolled hypertension among South African adult residents of Mkhondo municipality. BMC public health. 2020 Dec;20(1):1-0. https://doi.org/10.1186/s12889-020-09174-7

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Masilela C, Pearce B, Ongole JJ, Adeniyi OV, Benjeddou M. Single Nucleotide Polymorphisms Associated with Metformin and Sulphonylureas' Glycaemic Response among South African Adults with Type 2 Diabetes Mellitus. Journal of Personalized Medicine 2021 Jan 11(2), 104.https://doi.org/10.3390/jpm11020104

Journal articles – under review

Masilela C, Xhakaza L, Pierce B, Ongole JJ, Adeniyi OV, Benjeddou M. Single Nucleotides Polymorphisms in Amlodipine associated genes and their associations with blood pressure control among South African adults with Hypertension. Pharmacogenetics and Genomic.

Masilela C, Pierce B, Ongole JJ, Adeniyi OV, Benjeddou M. Association of Five Single Nucleotide Polymorphisms with Enalapril Treatment Response among South African Adults with Hypertension. Medicine.

Journal article – Not included in this thesis

Masilela C, Xhakaza L, Pierce B, Ongole JJ, Adeniyi OV, Benjeddou M. Social and clinical determinants of obesity among South African adults with multi-morbidities. BMC Public Health.

Masilela C, Adeniyi OV, Benjeddou M. Prevalence and determinants of dyslipidaemia among South African adults with multi-morbidities. Scientific Reports.

Abbreviations

ABCC8   ATP-binding cassette transporter sub-family C member 8

ADD1   Alpha-adducin

ADP   Adenosine diphosphate

ADRB2   Beta-2 adrenergic receptor

AKT   Protein kinase B

ATP   Adenosine triphosphate

CACNA1C   Voltage-gated calcium channel α1C

CAPN10   Calpain-10

CCB   Calcium channel blocker

CPA6   Carboxypeptidase A6

CPA6   Carboxypeptidase A6

DAG   Diacylglycerol

DM   Diabetes Mellitus

eNOS   Nitric oxide synthase

GWAS   Genome Wide Association Studies

HbA1c   Glycated hemoglobin

KCC1   potassium chloride cotransporter

KCNJ11   Potassium Inwardly Rectifying Channel Subfamily J Member 11

MDR	Multifactor dimensionality reduction
NBEA	Neurobeachin
NCDs	Non-communicable diseases
NO	Nitric Oxide
OCTs	Organic cation transporters
PI3-K	Insulin receptor substrate-1
PRPF31	Pre-mRNA processing factor 31 homolog
RAS	Renin-angiotensin system
SCNN1B	Sodium channel epithelial 1 subunit beta
SLC	Solute carrier
SNP	Single nucleotide polymorphism
SP1	Specificity protein 1
SU	Sulfonylurea
SUR	Sulfonylurea receptor
SUR1	Sulfonylurea receptor 1
T2DM	Type 2 diabetes mellitus
VEGFA	Vascular Endothelial Growth Factor A
WHO	World Health Organisation
WNK1	Protein kinase

List of Figures

List of tables

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Preface

The study protocol was approved by the Research Ethics Committee of the University of the Western Cape. The Mpumalanga and Eastern Cape Department of Health, Piet Retief hospital and Cecilia Makiwane Hospital clinical governance gave permission for the implementation of the study protocol across the four sites of the Mpumalanga and Eastern Cape Province. The research process followed the Helsinki Declaration and the rights of individuals to privacy and confidentiality were respected throughout the period of the study. This thesis is comprised of nine chapters arranged in the following order: Chapter 1 (introduction), Chapter 2 (literature review), Chapter 3-8, (studies conducted in article-ready format) and Chapter 9 (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

Table of Contents

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Chapter 1 – General Overview

1.1 Introduction

Hypertension has been declared a global public health emergency. Furthermore, the diseases often co-exist with DM and its prevalence doubles in individuals with DM than in the general public [1]. In the presence or absence of DM, hypertension is frequently associated with a variety of metabolic complications, including cardiovascular diseases which account for majority of pre-mature morbidity and mortality cases world-wide [1]. Morbidity and mortality can be reduced through secondary prevention strategies that involve regular screening for early detection of the disease and appropriate treatment to avoid complications [2,3]. To control the disease it is necessary to determine associated risk factors [3]. It is also important to manage these risk factors to prevent or delay the occurrence of life threatening complications [2,4]. Improvements in disease management and monitoring relies on epidemiological data that clearly states the prevalence, clinical and local influential factors [5]. Despite this knowledge, the prevalence of uncontrolled hypertension remains unknown, particularly in the rural areas of South Africa. Furthermore, the socio-demographic and clinical risk factors that influence the progression and control of the disease among these communities are understudied. We carefully designed a cross-sectional study that was comprised of 329 individuals, from whom we comprehensively assessed the prevalence and factors associated with uncontrolled hypertension in individuals receiving care in primary healthcare facilities based in the rural areas of Mkhondo Municipality (Mpumalanga Province, South Africa) (**Chapter 3**). In addition to clinical and socio-demographic factors, the risk of cardiovascular diseases and kidney disease, associated with hypertension, can be alleviated by the use of anti-hypertensive drugs (6). However, less than 50% of individuals with hypertension are adequately controlled

1

[7]. In selected cases, clinical algorithms based on the estimation of comorbidity or chemical variables such as plasma renin levels and lipid profile may be helpful in stratifying patients who may benefit from treatment. Furthermore, variability in drug response brought by genetic polymorphisms has been noted as a contributor towards the high prevalence of uncontrolled hypertension [7,8]. A growing number of clinical and observational studies have demonstrated that SNPs contribute approximately 30% of inter-individual variability in drug response and efficacy [9,10]. As such, the majority of patients living with hypertension remain uncontrolled, even with the addition of multiple classes of therapeutic drugs [9]. This realisation has led to a new field of study known as Precision Medicine. Analysis of genomic data is a key component of Precision Medicine and has significant potential to inform clinical care for individuals with hypertension [11]. However, one potential limitation of genomic medicine is the under-representation of African populations in genomics research, more especially in research that concerns anti-hypertensive drugs. To bridge this knowledge gap, we used MassArray to genotype DNA samples from 291 hypertensive individuals belonging to the Nguni tribe of South Africa. We investigated the level of association of nineteen SNPs with blood pressure control among adult patients treated with hydrochlorothiazide (**Chapter 4**). In **Chapter 5**, we assessed the level of association between twelve SNPs with an uncontrolled blood pressure for adult patients treated with amlodipine, using DNA samples collected from 304 hypertensive individuals belonging to the Nguni tribe who were undergoing continuous amlodipine therapy. **Chapter 6** examined the association of five SNPs in selected genes (ABO, VEGFA, BDKRB2, NOS3 and ADRB2) with blood pressure response to enalapril treatment. We further assessed genetic interactions that exist within these genes and their implications in enalapril treatment response in 284 participants belonging to the Nguni tribe of South Africa on continuous treatment for hypertension.

Diabetes Mellitus is one of the leading causes of mortality in modern society [12]. Similar to hypertension, uncontrolled DM is associated with detrimental effects including kidney and cardiovascular diseases [13,14]. Intensive programs, that consider socio-demographic factors aimed at reducing the incidence of cardiovascular diseases in individuals with DM, have proven to be of moderate efficacy in the developed world [15]. Furthermore, the influencing factors of uncontrolled DM are important in crafting context-specific interventions toward improving the clinical outcomes of people with DM. However, the extent in which uncontrolled DM affects the rural communities of South Africa as well as its local influencing factors are unknown. The present study fulfills the missing gaps by describing the socio-demographic and clinical profiles of individuals with DM, and further determines the rate and influencing factors of glycaemic control among adult residents of Mkhondo Municipality attending chronic care for DM (**Chapter 7**). Moreover, when lifestyle changes are not sufficient to ameliorate the clinical features of DM, particularly T2DM, it is necessary to design an appropriate pharmacological approach that includes multiple oral anti-diabetic drugs [16]. However, a number of studies have shown a widespread variability in glycaemic response in patients treated with similar anti-diabetic drugs [17]. Specifically, a considerable amount of the genetic variability observed in T2DM patients has been found in genes directly or indirectly implicated in the activity of oral anti-diabetic drugs [17]. Little is known about the influence of SNP on glycaemic response to metformin and SU combination therapy among individuals of African origin. In addition to non-genetic influencing factors, we extended the study to examine the association of polymorphisms belonging to *SLC22A1, SP1, PRPF31, NBEA, SCNN1B, CPA6 and CAPN10* genes with glycaemic response to metformin and SU combination therapy among South African adults with T2DM (**Chapter 8**). The main conclusions of the study, and a few prospects of the future of pharmacogenomics and Precision Medicine in South Africa, are summarised in **Chapter 9.**

3

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Chapter 2 – Literature Review

2.1 Search strategy

A search for published population-based surveys and cross-sectional studies, reporting on the prevalence of Hypertension and DM, pathophysiology of both diseases, as well as polymorphisms associated with blood pressure and glycaemic control in response to anti-hypertensive and anti-diabetic drugs across different populations was conducted via the University of the Western Cape online library. Google Scholar was also utilised to locate open access articles and books. Selected articles were written in English and indexed in PubMed, Scopus and Web of Science. Articles published between year the 2010 and 2020 were prioritised. However, landmark publications such as those reporting on the pathophysiology of both diseases were included irrespective of the year of publication. The following search terms were used to locate articles specific to this study: *prevalence of diabetes mellitus, prevalence of diabetes*, *uncontrolled hypertension*, *uncontrolled diabetes*, *single nucleotide polymorphisms*, *genetic variability, anti-diabetic drug response, anti-hypertensive drug response, hydrochlorothiazide treatment response, amlodipine treatment response, metformin treatment response, enalapril treatment response* and *non-communicable disease*. Variations of these terms were used to ensure exhaustive search results.

2.2 Introduction

Non-communicable diseases (NCDs) are described as chronic diseases, non-infectious and running a long course [1]. NCDs evolve as a result of complex interaction of genetic, physiological, environmental and behavioural factors [2]. The main types of NCDs are cardiovascular diseases (hypertension, coronary artery disease and stroke), cancers, chronic respiratory diseases (chronic obstructive pulmonary disease and asthma) and diabetes mellitus (DM). NCDs account for over 40 million deaths world-wide. With DM, cancer, cardiovascular

6

diseases and chronic lung diseases making the top four of the leading causes of death globally [3]. Furthermore, there has been a surge in NCDs in sub-Saharan Africa (SSA) that is driven by the increasing prevalence of cardiovascular risk factors such as hypertension, obesity, diabetes, dyslipidaemia, unhealthy diets, harmful use of alcohol and reduced physical activity [4]. NCDs are predicted to overtake communicable, maternal, neonatal and nutritional (CMNN) diseases combined as the leading cause of mortality in sub-Saharan Africa by 2030. This will cause a major impact on the economic productivity and also threaten the sustainability of healthcare systems in this region [4]. Important efforts are therefore needed to reduce the burden of NCDs in SSA, starting with the provision of reliable epidemiological data to appropriately inform prevention and treatment strategies that are guided by pharmacogenomics techniques.

Pharmacogenomics is a relatively new field of study that has been designed for understanding how genes influence an individual's response to drugs [5]. The focus of pharmacogenomics is the identification of genetic variants that influence drug efficacy and safety, mostly through alterations in pharmacokinetics and pharmacodynamics. Furthermore, pharmacogenomics takes advantage of the current advances in genome characterisation technology, as well as bioinformatics, which enable decoding of genetic variants affecting populations with specific drug response phenotypes [6]. For the pharmaceutical industry, pharmacogenomics may improve the drug development process through faster and safer drug trials. Individuals who are likely to benefit or experience adverse drug reactions could be detected long before the drug is commercialised [7]. This will enable the realisation of an emerging approach for disease treatment and prevention known as Precision medicine. Precision medicine takes into account an individual's genetic variability, environmental and lifestyle factors in order guide drug selection and dosing. This could limit patient exposure to medication that could be harmful or non-beneficial [8,9]. The limitation of precision medicine in Africa is the underrepresentation

7

of African-specific genomic data [9]. For precision medicine to become a reality in sub-Saharan Africa, more genomic data applicable to indigenous populations is needed.

2.3 Prevalence of Hypertension

The prevalence of hypertension continues to be an important health concern in the developing and developed world [10,11]. Hypertension affects over 1 billion people and accounts for 9.4 million annual deaths world-wide. This makes hypertension the single most important cause of morbidity and mortality globally [12]. Furthermore, nearly a third of the people living with hypertension reside in low- and middle-income countries, particularly those in the African continent where health resources are scarce and stretched by a high burden of infectious diseases [13]. In the year 2000, there were approximately 80 million adults with hypertension in sub-Saharan Africa. Based on the current epidemiological data, the number of people with hypertension is expected to increase to 150 million by the year 2025 [13]. According to the World Health Organisation, approximately 27.4% of men and 26.1% of women in South Africa have hypertension [14]. Literature suggests that 1 in 3 South African adults have high blood pressure, which is responsible for 1 in 2 strokes and 1 in 5 heart attacks that occur. Surveys conducted in the country have also revealed that hypertension is often not diagnosed and, when diagnosed, is often inadequately treated [11,14,15]. Using data from the South African National Health and Nutrition Examination Survey (2011–2012), Berry et al. showed that only 13.5% of hypertensive patients undergoing treatment were controlled, whilst in the rural areas of the country, figures as high as 75% were reported [15]. New systems in health facilities that are aimed at improving awareness, hypertension care and adherence to anti-hypertensive medication should be established in order to improve outcomes of care for people with hypertension.

2.4 Pathophysiology of hypertension

Hypertension is defined as a complex condition characterized by systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥90 mmHg [15,16]. Metabolic features of hypertension include increased salt sensitivity and low plasma renin activity, elevated triglycerides, reduced high-density lipoprotein (HDL) cholesterol, glucose intolerance and insulin resistance [17,18]. Hypertension usually does not present any symptoms; however, it has been associated with an increased risk of coronary artery disease, stroke, heart failure, peripheral vascular disease, loss of vision and chronic kidney disease [13,16,19]. Although hypertension is an area of intensive research, its pathophysiology is very complex and remains a subject of debate. A number of complex physiological mechanisms are involved in the maintenance of normal blood pressure, and changes in these processes may lead to hypertension [20]. There are many inter-related factors which contribute to raised blood pressure, including endothelial dysfunction, renin-angiotensin system (RAS) and insulin resistance; and their roles may differ between hypertensive patients [20–22,22].

2.5 Endothelial dysfunction

The endothelium is the innermost layer of the blood vessels that forms an interface between circulating blood and the vessel wall (21). The biological role of the endothelium is to regulate vascular tone, permeability, cell growth and the interaction between the leukocytes, thrombocytes and the vessel wall [21,23]. Under normal circumstances, the endothelium is naturally in a vasodilator resting state as a result of nitric oxide (NO) activity. Nitric Oxide is a soluble gas that is produced from L-arginine amino acid in endothelial cells by the nitric oxide synthase (eNOS) in response to stimuli including mechanical stress [24,25]. Nitric oxide diffuses through the underlying smooth muscle tissue thereby stimulating guanylate cyclase. In turn, guanylate cyclase stimulates an increase in cyclic GMP (cGMP) production, thus, triggering vasodilation [24,26]. Any damage to the endothelium (endothelial dysfunction) can

9

cause impairment of its normal function, including endothelium-dependent vasodilation and vasoconstriction. Endothelial damage is believed to be the earliest manifestations of vascular dysfunction in obese individuals and smokers, and precedes hypertension [24,27,28]. There are multiple mechanisms that are involved in endothelial dysfunction; however it appears that reduced NO production is a key regulator. Low NO levels may be a result of decreased endothelial nitric oxide synthase expression or reactive oxygen species (ROS) that convert NO to peroxynitrite, a substance with no vasodilatory effects [24,26]. Also, it can occur through the inhibition of NO by endothelium derived contracting factors [24,29]. Apart from persistently elevated blood pressure, endothelial dysfunction is a characteristic of cell proliferation, platelet activation, vascular permeability, and a pro-inflammatory and pro-thrombotic phenotype, including leucocyte-endothelial interactions that participate in vascular inflammation and increased adhesion and aggregation of platelets [21].
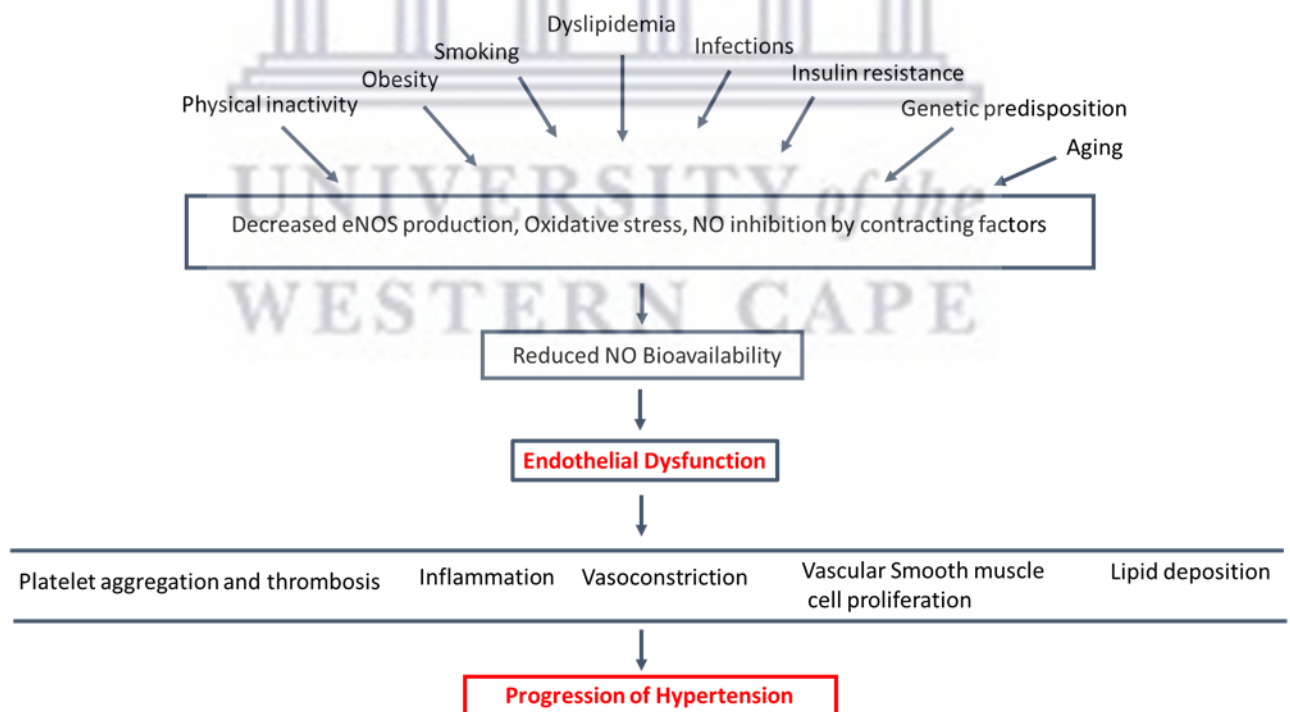
Figure 2.1: An illustration of the different factors that influence endothelial function and its progression to hypertension. Figure adapted from Beevers et al. [20]; Savoia et al. [21]; Konukoglu and Uzun [23]; Bleakley et al. [26] and Lüscher et al. [29].

2.5 Renin-angiotensin system

The renin-angiotensin system (RAS) is a hormone system that regulates blood pressure, fluid and electrolyte balance, as well as systemic vascular resistance [22]. When renal blood flow is low or when salt delivery to the distal convoluted tubule is decreased, renin from the juxtaglomerular apparatus of the kidney is secreted directly to the circulatory system. Plasma renin converts angiotensinogen released by the liver to angiotensin-1. This is subsequently converted to angiotensin-2 by the angiotensin converting enzyme (ACE) that is predominantly expressed on the surface of endothelial cells in the pulmonary circulation [22]. Angiotensin-2 is a potent vasoconstrictor that causes an increase in blood pressure. In the proximal convoluted tubule of the kidney, angiotensin-2 acts to increase Na-H exchange. Increased sodium concentration increases blood osmolarity, leading to a shift of fluid in blood volume and extracellular space (ECF), consequently increasing arterial blood pressure [30]. In the zona glomerulosa, angiotensin-2 has been shown to stimulate the production of aldosterone from the adrenal gland, resulting in increased sodium reabsorption and potassium excretion at the distal tubule and collecting duct of the nephron, subsequently increasing blood volume and ECF [20,22,30]. This system serves a critical function; however,

it can be activated inappropriately leading to persistently elevated blood pressure. For instance, renal artery stenosis results in a decreased volume of blood reaching the kidneys. Therefore, the juxtaglomerular cells sense a decrease in blood volume, activating RAS. This can lead to an inappropriate elevation of circulating blood volume and arteriolar tone due to poor renal perfusion [30]. In some cases, RAS can be activated by high dietary salt intake and Reactive Oxygen Species (ROS) [31,32]

2.6 Insulin resistance

Insulin resistance is usually associated with type 2 diabetes mellitus (T2DM) [33,34]. Furthermore, it has been strongly associated with vasoconstriction and vascular proliferation, suggesting that insulin resistance may play a role in endothelial dysfunction and the progression of hypertension [35]. In a healthy state, insulin promotes vasodilation by increasing NO production through a mechanism that involves insulin receptor substrate-1 (IRS-1), phosphoinositide 3-kinase (PI3-K) and protein kinase B (AKT). Insulin receptor substrate-1 interacts with PI3-K and activates AKT, which subsequently phosphorylates eNOS and stimulates NO production [36]. Inhibition of insulin induces IRS-1 activation, leads to reduced NO bioavailability and impairs vascular relaxation. *In vivo* studies have shown that insulin resistance may also lead to up regulation of the Endothelin 1 (ET-1) pathway, which diminishes NO bioavailability and contributes to increased vascular constriction [37]. In obese patients, as well as murine models of selective knockouts of the insulin receptor (IR), increased tubular absorption of sodium was observed [38]. Increased tubular secretion of sodium promotes a compensatory shift in the pressure natriuresis curve towards higher blood pressure. These effects on sodium and pressure natriuresis may be a result of an increase in adipose tissue mass and extracellular matrix accumulation [19]. This suggests that obesity may be the link between insulin resistance and endothelial dysfunction.

2.8 Renal sympathetic nervous system

Renal sympathetic nervous system (RSNA) plays an important role in the regulation of renal excretory function and blood pressure. The activity of RSNA has been observed in both hypertensive human and animal models of hypertension [39,40]. Even so, the mechanisms underlying these processes remain unclear. However, recent studies revealed the activation of three pathways in salt-sensitive hypertension which include: (1) an increase in tubular reabsorption of urinary sodium and water, (2) a reduction of renal blood flow and glomerular

12

filtration rate (GFR) and (3) the release of renin from the juxtaglomerular apparatus, thereby activating the renin–angiotensin–aldosterone cascade [39]. The three pathways are based on the activation α1- adrenergic receptor and β1- adrenergic receptor (ADRB1). Alpha 1-adrenergic receptors are located on the renal vasculature, nephrons and proximal tubules where they play a substantial role in vasoconstriction and sodium reabsorption [41]. On the other hand, renal β1-adrenoreceptors are located on the juxtaglomerular cells, nephrons, distal tubules and collecting ducts, where they stimulate renin secretion and suppress potassium secretion. The stimulation of β-adrenoreceptors mediates reabsorption of calcium (Ca+) and magnesium (Mg+) ions in the cortex, and of sodium chloride (NaCl) in the medulla, through the activation of cyclic AMP (cAMP) (40) . Thus, a sustained overactivity of RSNS has been linked with the development of hypertension, organ damage including cardiac hypertrophy and kidney failure [40,42].

2.9 Factors associated with hypertension control

2.9.1 Physical activity

Physical exercise is defined as voluntary body movement that is produced by the skeletal muscle resulting in energy expenditure [43]. Regular physical activity is a key component of lifestyle therapy for the prevention and treatment of hypertension [43–45]. The World Health Organization (WHO) recommends 150 minutes of moderate-intensity aerobic physical activity per week for adults aged between 18 and 64 years [45]. Several studies consistently demonstrated that engaging in physical activity reduced both systolic and diastolic blood pressure by as much as 5-7 mmHg [46]. It was further demonstrated that mortality risk was higher among inactive hypertensive patients [44]. The reduction in blood pressure is perceived to be due to attenuation in peripheral vascular resistance, as a result of neurohormonal and structural responses with reductions in sympathetic nerve activity and an increase in arterial

13

lumen diameters, respectively [47]. Furthermore, it has been proposed that blood pressure response to physical activity may be due to favorable changes in oxidative stress, inflammation, endothelial function, arterial compliance, body mass, renin-angiotensin system activity, parasympathetic activity, renal function and insulin sensitivity [46]. It is important to note that the blood pressure lowering effect of physical activity is highly variable. Different exercise regimes, environmental factors and genetic factors may be responsible for the variability that has been observed in blood pressure response to exercise.

2.9.2 Diet

Diet based approaches are recommended as first-line therapy for the prevention and control of hypertension. A recent clinical trial showed that a diet consisting of fruit, vegetables, low-fat dairy products, whole grains, poultry, fish and nuts, and is reduced in fat, red meat, sweets and sugar-containing beverages, in the absence of weight loss or sodium restriction, significantly lowered BP [48]. Furthermore, reducing dietary sodium intake lowered systolic blood pressure by 3.8 mmHg in adults. The study further demonstrated that a larger increase occurred among older adults than in younger persons. A small clinical trial conducted among individuals with resistant hypertension showed that reducing sodium intake by approximately 4.5 g/day, lowered systolic and diastolic blood pressure by 22.7 and 9.1 mmHg respectively [49]. In addition to blood pressure lowering effects, studies have shown that reducing sodium intake lowered the incidence of hypertension-related complications. Law et al. estimated that a reduction of daily salt intake by 3g may reduce the incidence of stroke by 22% and of ischemic heart disease by 16% in a Western population. It was further estimated that lowering salt content in processed food products may lower blood pressure by at least twice as much and prevent the incidence of hypertension-associated complications [50].

2.9.3 Smoking

Smoking can increase the risk of hypertension, myocardial infarction and kidney failure. Furthermore, smoking induced hypertension can not only reduced life expectancy, it can also reduce the quality of life [51,52]. Over five million people in the world die from smoke-related diseases [53]. The mechanism in which smoking induces hypertension is linked to RAAS [54]. Tobacco products consist of nicotine, a substance that acts as an adrenergic agonist that enhances local and systemic catecholamine release. Also, nicotine has been shown to stimulate the release of vasopressin, a hormone that is responsible for increasing peripheral vascular resistance and arterial blood pressure [53,54]. Despite these observations, paradoxical associations between current smoking and systolic blood pressure, uncontrolled blood pressure and isolated uncontrolled systolic blood pressure were shown among hypertensive patients. Several epidemiological studies have shown that habitual smokers generally have lower blood pressure or the same as non-smokers [51–53]. On the other hand, a study conducted by McNagny et al. showed that individuals with uncontrolled hypertension were more likely to be current tobacco users or have a history of tobacco use. Moreover, individuals in this sub-group were also more likely to be less compliant to medication [52]. The differences observed may be due to sample size, study design, population background and adjusted factors used to control for confounding errors. The relationship between smoking and lower blood pressure remains elusive, but might become apparent with additional studies with a larger sample size.

2.9.4 Alcohol consumption

In the present day, alcohol beverages are consumed regularly by most of human societies in the world [55,56]. Current research suggests that the moderate consumption of alcohol is beneficial to the cardiovascular system and lowers the blood pressure [57]. However, excessive alcohol consumption is a major public health concern [55]. Epidemiological, as well as clinical studies have established the relationship between hypertension, risk of cardiovascular diseases

15

and excessive alcohol consumption [56]. There are several mechanisms that have been linked to alcohol-induced hypertension. For instance, alcohol can alter serum levels of vasoactive substances such as renin-aldosterone, and also cause a shift in the binding of the calcium ion (Ca^{2+}) in arterial and arteriolar smooth muscle cells, leading to increased sensitivity to endogenous vasoconstrictors [55]. It is also possible that alcohol ingestion induces high blood pressure by decreasing NO in the vascular endothelium, either due to inhibition of eNOS or endothelial damage as a result of inflammation [55,58]. Studies have reported increased sympathetic nervous system activation and discharge of sympathetic amines after alcohol consumption [59]. Others have reported that alcohol initiates central as well as peripheral reactions which in a synergistic manner induce hypertension [60].

Population studies have identified a strong association between excessive alcohol consumption and uncontrolled hypertension. It was further demonstrated that men who engaged in excessive drinking had 1.34-fold increase in the odds of poor blood pressure control [61]. A study conducted among Japanese individuals demonstrated that the systolic and diastolic blood pressure of hypertensive heavy drinkers was 2.3/20 mmHg higher than that of non-drinkers [62]. It was also shown that alcohol dependency was common among hypertensive men (20.6%) and women (16.7%) aged between 40-65 years [63]. Meta-analysis of 15 randomized controlled trials, in which alcohol reduction was the only intervention between active and control groups, found that reducing alcohol consumption among patients lowered systolic and diastolic blood pressure in a dose dependent manner [64]. Furthermore, a South African study showed hypertensive women were at a higher risk for harmful alcohol use. These findings suggest that excessive alcohol consumption is common among hypertensive patients. Thus, reducing alcohol consumption should be recommended as an important component for the management of hypertension among alcohol consuming patients.

16

2.9.5 Age

In humans, aging is a progressing process that results in decreased physiological function across all organs, leading to vulnerability to infections and diseases which dramatically increase the risk of morbidity and mortality. Data collected over a period of 30 years suggest that the prevalence of hypertension increases with age [65]. The risk of coronary artery disease, congestive heart disease and chronic kidney insufficiency is also high among older adults [66]. Notably, older adults account for a large proportion of hypertension-related mortality. Literature suggest that 70% of older adults have hypertension, compared to only 32% of adults aged 40-59 years [67]. While in Africa, an estimated 55% of older adults have been diagnosed with hypertension [68]. A population-based survey reported a 77.3% prevalence of hypertension among South African adults who were 50 years and older. It was further demonstrated that the prevalence of hypertension among this group differed by sex, with older women showing a higher rate of hypertension than men [69]. It was recently demonstrated that the prevalence of uncontrolled hypertension increases with age [70]. Furthermore, results from the population-based KORA-age 1 study showed that only 53.7% of older adults undergoing anti-hypertensive treatment had their blood pressure controlled, and the remainder had uncontrolled blood pressure [71]. A population-based study conducted among older adults in South India reported a 46.2% prevalence of uncontrolled hypertension [72]. A similar trend was observed among Chinese patients [68]. In Northern Ethiopia, the prevalence of uncontrolled hypertension was estimated at 48.6% among older adults [73]. While in South Africa, only 17% of patients who were treated for hypertension had controlled blood pressure [74]. There are numerous challenges that exist in the treatment of hypertension among this group of individuals, including altered drug metabolism, co-morbidities, increased blood pressure variability and orthostatic hypotension that makes it difficult to obtain tailored guidelines for treatment [67] It appears that there is no general principle for treating

hypertension among older adults and it increasingly becoming evident that the therapeutic strategy of one size fits all cannot be applied among the elderly because of the enormous functional heterogeneity that they present.

2.9.6 Sex

It has been suggested that sex may influence the prevalence and control of hypertension [75] . A number of cross-sectional studies suggest that the prevalence of hypertension is higher among men across all ethnicities in comparison to their female counterparts [75] . Some studies demonstrated that men have a much greater prevalence of hypertension from 20 until 65 years. Whilst more women over the age of 75 were hypertensive. It is also believed that women between the ages of 20-44 years are relatively protected from hypertension when compared with men in the same age group [76]. Observed sex differences in hypertension are due to biological and behavioural factors [77]. Biological factors include sex hormones, chromosomal differences and other biological sex differences that are protective against hypertension in a particular gender. Behavioural factors include smoking, alcohol consumption, low physical activity and high body mass index (BMI). It has been shown that men and women differ in these key behavioral risk factors and that they play an important role in hypertension control. Choi et al. [78] showed that the prevalence of hypertension was high among women who were obese and in men who consumed alcohol. The authors further demonstrated that the overall rate of control of hypertension was higher among younger women (51.3%) than in men (44.8%). Furthermore, Brazilian women diagnosed with hypertension showed better hypertension control in comparison to men [79]. In contrast, Ong et al. showed that there were no sex differences in blood pressure control among American patients. However, the study demonstrated that Central obesity, elevated total cholesterol level and low high-density lipoprotein cholesterol were significantly higher among women [78].

2.9.7 Race/Ethnicity

Race/Ethnicity reflects differences in social and cultural influences such as health behaviours, access to health care, and environmental exposures that may affect blood pressure [80]. A recent study showed that hypertension is more prevalent among African American (60%) in comparison to Caucasian (38%), Hispanic (42%) and Chinese patients (39%). It was further demonstrated that Caucasian participants had a lower rate of uncontrolled hypertension in comparison to all the study groups [81]. Despite receiving combination therapy, Gu et al. showed that African American patients were less likely to exhibit controlled blood pressure in comparison to Hispanics and Caucasians. This was consistent with a previous finding where African American participants showed a more aggressive form of hypertension [82]. It was further demonstrated that younger participants of Hispanic and African origin were approximately 40% less likely than Caucasians to achieve hypertension control [83]. The authors listed awareness and understanding of the disease, adherence to prescribed antihypertensive drugs, lifestyle changes and access to medical care, including drug affordability and biology of the disease, as the main factors associated with the differences in blood pressure response between different races/ethnic groups. Of note, these factors are strongly influenced by the level of education, financial status as well as cultural and social environment that determines disease awareness, social support and patient–physician interaction [83].

Racial/Ethnic disparities in blood pressure control may be a result of genetics. For instance, people of African origin are believed to retain more sodium and water. This notion is based on the fact that most African populations were hunter-gatherers who lived in a hot and arid environment where sodium was scarce [84]. These populations were in a critical sodium balance and any impairments in sodium reabsorption may have resulted in loss of circulatory homeostasis, which might have led to selection of genes that retained more sodium [85]. For

instance, a mutation in aldosterone synthetase may lead to a greater synthesis of aldosterone and protect an individual from sodium depletion [85]. In a healthy state, aldosterone synthetase promotes the production of aldosterone [85]. In turn, aldosterone stimulates the renal tubular epithelial sodium channel (ENaC) to reabsorb sodium in response to sodium depletion through activation of the RAAS [85–87]. The activity of ENac is regulated by neural precursor cells expressing developmentally down-regulated protein 4 (NEDD4) [88]. Alterations in the gene that encodes NEDD4 may result in limited degradation of ENaC and a greater expression in the cell surface in response to aldosterone. Aldosterone production in response to low sodium levels, acting together with increased ENaC activity, may enhance the ability to retain sodium and fluids [85]. It was recently demonstrated that people of African origin possess a genetic mutation on the genes that encodes aldosterone synthetase (*CYP11B2*) [89]. This may be the explanation for the aggressive form of hypertension that is observed among people of African origin.

Literature suggests that people of African origin are more likely to present a Liddle syndrome phenotype [85]. The main feature of this syndrome is overactivity of ENaC where both renin and aldosterone are suppressed [90]. Studies conducted in South Africa and the United States of America showed that renin and aldosterone was lower among Blacks than in Caucasians despite comparable sodium intake, suggesting an underlying genetic factor [91]. The association of specific mutations in genes that encodes ENaC (SCNN1B) with low renin and aldosterone, were described among people of African origin. Furthermore, a novel mutation (p.Arg563G) on SCNN1B was present among Black South Africans, including those of Nguni (Zulu and Xhosa), Sotho and mixed ancestry [92–94]. This mutation was associated with low-renin, low-aldosterone hypertension (Liddle phenotype), hypertension in kindreds and pre-eclampsia. This mutation was not observed among West Africans [92,94]. The mutation might have originated from the San people (original hunter-gatherers) who lived in hot and arid

conditions in Southern Africa [85]. In addition to the Liddle syndrome phenotype, people of African origin are more likely to exhibit primary aldosteronism as a consequence of bilateral adrenocortical hyperplasia [92]. A study conducted among Africans showed that patients with the primary aldosteronism phenotype had variants of aldosterone synthetase [85]. These variants may hinder efforts to control blood pressure among African hypertensive patients.

2.9.8 Dyslipidaemia

Abnormalities in serum lipid and lipoprotein levels (dyslipidemia) are recognized as major modifiable cardiovascular disease risk factors and have been identified as independent predictors for hypertension [95,96]. It has also been shown that the presence of dyslipidaemia worsens hypertension, and that lipid levels rise as blood pressure increases [97]. The mechanism by which dyslipidemia induces or worsens hypertension is not completely understood. However, evidence suggest that dyslipidaemia adversely affects the functional and structural properties of arteries and promotes atherosclerosis. These changes may impair blood pressure regulation which, in turn, predisposes individuals with dyslipidemia to hypertension or uncontrolled hypertension [98].

From an epidemiological perspective, hypertensive patients with co-morbidity of dyslipidemia are eighteen times more likely to develop cardiovascular diseases [99]. It was also demonstrated that patients with uncontrolled hypertension had high levels of low density lipoprotein cholesterol (LDL-C), even though they were receiving more anti-hypertensive drugs in comparison to patients with controlled hypertension [100]. Yan et al. reported that Chinese individuals who failed LDL-C targets were more likely to exhibit uncontrolled hypertension [101]. Furthermore, a study conducted among Nigerian men and women showed that patients with uncontrolled hypertension had higher levels of LDL-C, total cholesterol (TC) and non-high density lipoprotein cholesterol (non-HDL-C) [102]. Clinical guidelines

recommend adding statins to anti-hypertensive therapies for further cardiovascular benefits among hypertensive patients presenting a high risk for cardiovascular diseases. However, literatures suggest that physicians' compliance with these recommendations and patients' adherence to anti-hypertensive therapy, as well as lipid-lowering treatments, remain poor. As a consequence, blood pressure remains poorly managed and the risk of hypertension-related complications is increased [103].

2.10 Hypertension treatment

Hypertension treatment has evolved over the last decade, with health officials recognizing that there is no threshold below which elevated blood pressure causes no threat to health [104]. Recent guidelines, including those of the South African hypertension society, make it clear that the threshold above which blood pressure should be treated to prevent long-term complications is 140/90 mm Hg [105]. However, the control of hypertension is complex and it requires the collaborative efforts of the patient, physician and the health system. Upon diagnosis, lifestyle modifications, which include moderate sodium restriction, weight reduction in the obese, decreased alcohol intake and an increase in exercise, are recommended for the management of hypertension [47,61,105]. When lifestyle modifications fail to improve blood pressure control or when hypertension is already at an advanced stage, drug therapy is introduced. The most commonly prescribed anti-hypertensive drugs are: hydrochlorothiazide, amlodipine, enalapril and atenolol [105].

2.10.1 Pharmacokinetics of Hydrochlorothiazide

Hydrochlorothiazide is a thiazide diuretic that has been clinically used for over 50 years [106,107]. The drug is indicated for adjunctive therapy to treat edema associated with congestive heart failure and hepatic cirrhosis. It is also indicated for the treatment of hypertension as a sole agent or in combination with other anti-hypertensive drugs.

Hydrochlorothiazide is well absorbed (65% to 75%) following oral administration [69] . The plasma half-life of the drug is reportedly between 6 to 12 hours, with plasma concentrations reaching their peak between one to five hours following oral administration [106]. Moreover, hydrochlorothiazide concentrations are linearly related to the administered dose. It has been shown that concentrations of hydrochlorothiazide are 1.6 to 1.8 times higher in whole blood than in plasma [108]. Hydrochlorothiazide is not metabolized and it is eliminated primarily through renal pathways. When administered with food, the bioavailability of hydrochlorothiazide is decreased by 10%, the maximum plasma concentration is reduced by 20% and the time to maximum concentration increases from 1.6 to 2.9 hours. The absorption of hydrochlorothiazide is said to be reduced among patients with congestive heart failure [109]. The 12.5 mg and 25 mg daily doses of hydrochlorothiazide have been shown to lower systolic blood pressure by 5 mmHg to 7 mmHg and the diastolic blood pressure by 4 mmHg to 5 mmHg over a 24-hour period [106]. In comparison to other classes of drugs, blood pressure reductions, induced by hydrochlorothiazide, are much lower than those induced by angiotensin-converting enzyme inhibitors, calcium channel blockers or beta blockers. However, it has been suggested that the effects of hydrochlorothiazide are more consistent and reliable in almost all populations [69,110].

2.10.2 Pharmacodynamics of Hydrochlorothiazide

Despite decades of being used as an anti-hypertensive agent, the mechanism of action of hydrochlorothiazide is poorly understood. Literature suggest that the drug inhibits the transport of sodium chloride by blocking the Na^+, Cl^- symporter, located in the luminal membrane of distal convoluted tubules that is responsible for moving Na^+ and Cl^- into the cell using free energy produced by Na^+, K^+ and ATPase [69,111]. In turn, more sodium is excreted in the kidney accompanied by fluid and a loss of potassium and bicarbonate [112,113]. This loss in volume leads to diminished venous return, increased renin release, reduced cardiac output and

23

decreased blood pressure [42,69,113,114]. The mechanism in which hydrochlorothiazide induces its long-term effect on blood pressure is unknown. However, it has been suggested that the long term use of hydrochlorothiazide appears to reduce blood pressure by decreasing peripheral resistance [112]. On the other hand, when the drug is administered acutely, it lowers blood pressure by promoting diuresis and increasing plasma volume. Laboratory studies suggest that the drug induces vasodilation by inhibiting the enzyme carbonic anhydrase, thereby desensitising the smooth muscle receptors to the rise in calcium or by preventing autoregulation in the renal system [106].

Although thiazide diuretics including hydrochlorothiazide are regarded as safe, literature suggest that treatment with any drug may be accompanied by unintended effects. For instance, hydrochlorothiazide is associated with hyperlipidaemia, hyperglycaemia, new-onset diabetes, hypokalemia mediated beta-cell destruction and stimulation of RAAS [115]. A recent clinical trial showed that patients who were treated with hydrochlorothiazide had decreased insulin sensitivity [116]. It was further demonstrated that patients with diabetes who were prescribed hydrochlorothiazide developed significantly higher hepatic fat deposition, which could be explained by the decrease in insulin sensitivity [116]. The drug is also associated with increased levels of serum uric acid, that was correlated with the risk for diabetes [117,118].

2.10.3 Pharmacokinetics of Amlodipine

Amlodipine is an oral dihydropyridine calcium channel blocker (CCB) that was officially approved for clinical use over 35 years ago [119,120]. The drug is indicated for the treatment of hypertension alone or in combination with other oral anti-hypertensive drugs. Other indications include chronic stable angina and prinzmental angina. In comparison to other anti-hypertensive drugs in the same class, amlodipine has the longest half-life of 30 to 50 hours [119] . Owing to its long half-life, amlodipine is dosed once per day, making it favorable for

patient compliance [119]. Upon diagnosis, a starting dose of 5 mg/day is recommended with a maximum daily dose of 10 mg. Lower doses are recommended for the elderly and those with hepatic failure. Amlodipine has a high bioavailability, ranging from 60% to 80%, with plasma concentrations rising gradually to peak six to eight hours following oral administration [121]. The drug is metabolised in the liver, and slowly eliminated over 40-60 hrs. Of note, the elimination of amlodipine is largely dependent on hepatic metabolism. As a result, individuals with liver cirrhosis demonstrate impaired elimination [120,121]. Moreover, renal insufficiency does not influence the disposition of the drug. The volume of distribution that is exhibited by amlodipine is 21 L/kg after intravenous administration and it exhibits a high degree of protein binding (98%). If amlodipine is discontinued, blood pressure generally returns to baseline over one week without imposing danger on the patient [120].

2.10.4 Pharmacodynamics of Amlodipine

Normally, contraction of vascular smooth muscle initiates the influx of calcium into the cell via voltage-dependent L-type calcium channels [119]. The calcium binds to intracellular calmodulin, which subsequently binds to and activates myosin light-chain kinase (MLCK). Thereafter, the MLCK phosphorylates myosin light-chain, ultimately leading to muscle contraction and vasoconstriction. Contraction of the vascular muscle is further amplified by calcium-induced calcium release from the sarcoplasmic reticulum [119,121]. This leads to decreased vascular cross-sectional area, increased vascular resistance, and increased blood pressure. Amlodipine and other CCBs induce their anti-hypertensive properties by blocking the voltage-dependent L-type calcium channels, thus inhibiting the initial influx of calcium into the cell [119,121]. Reduced levels of calcium lead to decreased vascular smooth muscle contractility, increased smooth muscle relaxation and resultant vasodilation, thus causing a decrease in blood pressure [119]. Furthermore, amlodipine also relieves angina by blocking coronary spasm and restoring blood flow in the coronary arteries [121].

Apart from lowering blood pressure and relieving angina, amlodipine has been associated with peripheral edema and pulmonary edema [119]. Advanced age, obesity and gender are all factors that pre-dispose patients to amlodipine-induced edema, that may be attributed to vasodilation rather than fluid retention [122]. Also, amlodipine should be used with caution among patients with congestive heart failure, as it may increase the risk of future cardiovascular events and mortality. Other reported side effects include dizziness, fatigue, headache, palpitations and nausea, although these are generally not bothersome enough to cause discontinuation of the drug [120]. Incidents of amlodipine-induced side effects appears to be low, even though a substantially higher rate of disease is consistently being reported in clinical practice [122].

2.10.5 Pharmacokinetics of Enalapril

Enalapril belongs to a class of drugs known as angiotensin-converting enzyme (ACE) inhibitors that were originally introduced for clinical use in the year 1981. Since then, this class of drugs has been extensively used for the treatment of hypertension [123]. Enalapril is also indicated for the treatment of heart failure, left ventricular dysfunction and chronic kidney disease [124]. Furthermore, the drug is administered as a maleate salt and it was designed as pro-drug to improve the systemic availability of enalaprilat (active ACE inhibitor), which is poorly absorbed in humans [125]. About 60% of the enalapril dose that is administered orally is absorbed and peak serum levels are reached in about an hour. Absorption of the active form of enalapril is unaffected by food. Enalapril is esterified in the body to form enalaprilat, which reaches peak serum concentration between three to four hours [126]. The serum concentration of enalaprilat is linearly dependent on dose and its bioavailability is about 40%. Moreover, less than 50% of enalaprilat is protein bound and it is excreted unchanged in urine and faeces. Although, traces of enalaprilat were detected in both faeces and urine, the primary route of

elimination appears to be renal. The renal clearance rate of enalapril and enalaprilat are 18 L/h and 8.1 to 9.5 L/h, respectively, and a terminal half-life of 30 to 35 hours. A steady state is reached between three to four hours of oral doses every 24 hours. The drug may accumulate in patients with impaired renal function. Since enalapril is esterified in the liver, patients with impaired liver function fail to convert the drug to its active form [127]. However, steady-state plasma concentrations of enalaprilat appear similar in patients with congestive heart failure and those with hypertension after repeated doses. In patients with non-complicated hypertension, enalapril doses of 10 to 40 mg/day reduce systolic and diastolic blood pressure by 15 to 20%, with adequate pressure control being achieved in about 50 to 75% of patients on monotherapy [28].

2.10.6 Pharmacodynamics of Enalapril

Enalapril exerts its blood pressure lowering effect by competitively inhibiting ACE, thus blocking the conversion of angiotensin-1 to angiotensin-2 [129,130]. This reduces arterial pressure, preload and afterload on the heart. Blocking angiotensin-1 may stimulate aldosterone production from the adrenal cortex, leading to an increase in Na^+ and water reabsorption, reduction in blood volume, venous pressure and arterial pressure [131,132].The vasodilatory effect of enalapril stems from its ability to stimulate bradykinin receptor (BR2), which in turn promotes nitric oxide (NO) production [133,134]. This effect may be attributed to enalapril's ability to inhibit the breakdown of bradykinin [135]. The vasodilatory effect of enalapril could also be a result of its ability to induce the expression of vascular endothelial growth factor (VEGF), through an interaction with BR2 and the angiotensin type 2 receptor, thereby stimulating NOS3 in vascular cells [136]. Furthermore, enalapril-induced NO production could also be stimulated by beta-2 adrenergic receptor (ADRB2) which is expressed in vascular endothelial cells. Beta-2 adrenergic receptor induces cyclic adenosine-3′, 5′-monophospate

(cAMP), which in turn triggers the NO system to activate vasodilatation by increasing arginine uptake [137].

Unlike other anti-hypertensive drugs, enalapril does not cause hypokalaemia, hyperglycaemia, hyperuricaemia or hypercholesterolaemia [138]. Furthermore, enalapril monotherapy was associated with a decrease in fasting blood glucose and HbA1c levels among patients with co-existing diabetes and hypertension [139]. The mechanism in which enalapril induces these effects is yet to be established. Moreover, reported adverse reactions associated with enalapril include headache, nausea, myocardial infarction, constipation, rash, fever and hypersensitivity reactions [125]. Hypotension is a classic manifestation of enalapril overdose; however, cases of enalapril-induced hypotension are rare [125]. Also enalapril's ability to potentiate bradykinin production and also inhibit its breakdown has been associated with persistent cough among patients undergoing therapy [125,132,140]. Overall, the drug appears to be well tolerated by patients, with only a few serious adverse effects reported [128].

2.10.7 Pharmacokinetics of Atenolol

Atenolol belongs to a class of drugs that is known as beta-blockers. Beta-blockers were first introduced for clinical use in the early 1960's. This class of drugs is indicated for the treatment of congestive heart failure, cardiac arrhythmias and hypertension [141]. Although, the popularity of beta-blockers has declined over the years, atenolol is historically one of the most frequently prescribed drugs since its approval for clinical use in 1969 [142]. Atenolol is available in 25 mg, 50 mg and 100 mg dosed tablets for oral administration or 0.5 mg/mL for intravenous injection, depending on the indication. The initial dosage for adults is 50 mg/day, given as a single dose or in combination with other anti-hypertensive drugs [143,144]. In elderly patients, presenting with renal impairment, a lower dose of 25 mg/day may be prescribed provided they have creatinine clearance of under 15 ml/min, or a maximum dose of

50 mg a day if the creatinine clearance is 15 to 35 ml/min. In cases of resistant hypertension, the dosage may be increased to a single 100 mg/day; however, any dosage higher than a 100 mg is unlikely to produce any therapeutic benefits [144]. Atenolol's duration of action is dose dependent, the effects are apparent within an hour and persist for 24 hrs. The effects of an intravenous dose of the drug are evident within five minutes but dissipate after 12 hours. Since atenolol is a hydrophobic drug, it has difficulties crossing cellular membranes and tends to be poorly absorbed and metabolised. Furthermore, only 50% of the drug is absorbed following oral administration [144]. The remainder is excreted in faeces unchanged. Atenolol is metabolised in the liver to a minimal extent, however only 90% of the substance is present in plasma following intravenous injection. In patients with normal renal function, the elimination half-life of atenolol is between six and nine hours following oral administration. In patients with renal impairment, the elimination half-life of atenolol gradually increases to 36 hours [144].

2.10.8 Pharmacodynamics of Atenolol

Atenolol is a cardio-selective beta-1-adrenergic antagonist that elicits its anti-hypertensive properties by selectively binding ADRB1 that is located in vascular smooth muscle and the heart [144). When activated, ADRB1 stimulates the production of intracellular cAMP. Thereafter cAMP activates protein kinase A (PKA), which phosphorylates membrane calcium channels leading to an increase in calcium entry into the cytosol and reuptake by the sarcoplasmic reticulum [145,146]. The increase in calcium loading leads to a positive inotropic effect. By blocking ADRB1, the positive inotropic effect and chronotropic actions of endogenous catecholamines such as isoproterenol, norepinephrine, and epinephrine, is inhibited. This series of events leads to the obstruction of sympathetic stimulation and reduction in heart rate, blood pressure and decreased myocardial contractility [44,147]. Moreover, the anti-hypertensive effect of atenolol may be attributed to action on the central

nervous system (CNS) or a possible inhibition of RAAS rather than a direct effect on vasculature.

Furthermore, atenolol has been extensively used as a fist-line anti-hypertensive agent for decades before it was downgraded to a fourth-line drug due to adverse reactions. Since sympathetic activity is necessary for maintaining cardiac function, the reduced contractility that is induced by atenolol may worsen heart failure [147]. Also, the drug has the potential to induce fatigue, depression, and sleep disturbances such as insomnia. The mechanism implicated in these events is unknown [148]. Other reported side effects include bronchospasm, bradycardia, diarrhoea, dizziness, constipation, confusion, visual impairment and vomiting. Unlike other drugs that are used in the treatment of hypertension, atenolol does not have intrinsic sympathomimetic or membrane stabilizing activity nor does it produce changes in glycaemic control [148].

2.11 Prevalence of Diabetes Mellitus

Diabetes Mellitus (DM) remains a global health problem affecting about 463 million adults between the ages of 20 and 79 years. Furthermore, the number of DM cases is expected to increase to 700 million by the year 2045 [149]. In the year 2016, an estimated 1.6 million deaths were directly caused by DM, whilst in the year 2012, over 2 million deaths were attributed to hyperglycaemia [150]. Approximately 50% of deaths that were attributed to hyperglycaemia in the year 2016, occurred before the age of 70 years. In addition, it has been shown that diabetes-related deaths that occurred prior to the age of 70 years were more prevalent in low-middle income countries [150]. Diabetes Mellitus was considered a rare disease in Sub-Saharan Africa [151]. However, in the last two decades, DM has emerged as an important non-communicable disease. Furthermore, the challenge posed by the high prevalence of DM in sub-Saharan Africa is overwhelming, as the region carries a double burden of communicable and

non-communicable, and is also challenged by scarce resources [152]. In the year 2017, the prevalence of DM was estimated at 15.9 million in this region. By the year 2045, the number of people living with DM is expected to exceed 40 million and outpace all other global regions. It has been suggested that as many as 69% of people residing in this region are living with undiagnosed DM. This has led to a high prevalence of diabetes-related complications including cardiovascular diseases and death [153]. The top five countries with the highest number of individuals living with diabetes are Nigeria, South Africa, Democratic Republic of Congo, Ethiopia and Tanzania [151]. According to the International Diabetes Federation, 3.85 million (7%) South African between the ages of 21-79 years have DM [154]. This is a dramatic increase from the 4.5% that was observed in 2010. Furthermore, the majority of DM cases that are observed in the country may be attributed to type 2 diabetes mellitus (T2DM). The overall prevalence of T2DM among South African adults is estimated at 12.8% [155], while studies conducted in different parts of the country have reported figures up to 82.8% [156]. Current estimates suggest that the prevalence of undiagnosed DM is 69% [157]. Therefore the documented prevalence of DM in South Africa is likely an underestimation, as the presented figures do not account for undiagnosed DM as well as unmet diabetes care. Currently, there is no record of the national prevalence of uncontrolled T2DM. However, studies conducted in the rural Eastern Cape Province estimated a prevalence of uncontrolled T2DM between 82 to 83.3% [158,159], whilst a glycaemic control rate of 27% was recorded in the Tshwane district of the Gauteng Province [160]. These figures do not entirely represent the large populations in different parts of the country; however they highlight the need to improve diabetes care and self-efficacy in the country.

2.12 The pathophysiology of Type 2 Diabetes Mellitus

Currently, there are five forms of DM, namely Type 1 (T1DM), T2DM, maturity-onset diabetes of the young (MODY) and gestational diabetes. Maturity-onset diabetes of the young is a rare

31

form of non-insulin dependent DM that is commonly observed in adolescence or young adults before the age of 25 years [161]. MODY is often misdiagnosed as T1DM, a chronic autoimmune disease characterised by insulin deficiency following the destruction of pancreatic beta-cells and resultant hyperglycaemia [162]. On the other hand, gestational diabetes is a form of DM that is diagnosed during pregnancy. Like any form of DM, gestational diabetes is characterised by chronically elevated blood glucose [163]. The most common form of DM is T2DM, defined as a complex metabolic disease characterised by chronic hyperglycaemia as a consequence of defects in insulin secretion, insulin action or a combination of both [33,164,165]. Type 2 diabetes mellitus is preceded by a number of complex metabolic conditions including oxidative stress, endoplasmic reticulum stress, dyslipidemia, hyperinsulinemia and subclinical inflammation, which eventually leads to insulin resistance [166–168]. Over time insulin resistance progresses to T2DM [33]. The pathophysiology of T2DM remains elusive, however; genetics, obesity as well as numerous lifestyle factors, including excessive caloric intake, physical inactivity, cigarette smoking and consumption of alcohol are considered to be important risk factors for the development of insulin resistance and its progression to T2DM [169,170].

Under normal conditions, insulin regulates hepatic glucose uptake and metabolism through activation of hepatocyte insulin receptors. These receptors decrease glucose production by activating hepatic glucose synthesis and through transcriptional down-regulation of gluconeogenic enzymes, predominantly by FOXO1 phosphorylation [166,171]. In the skeletal muscle, insulin binds the intracellular α subunit of the insulin receptor and triggers tyrosine phosphorylation of the intracellular domain of the β subunit. Thereafter, the insulin receptor phosphorylates insulin receptor substrate (IRS)-1 on tyrosine residues. These lead to a series of events that trigger the translocation of glucose transporters to the plasma membrane and increased glucose transport into the cell [172]. In adipose tissue, insulin stimulates glucose and

free fatty acid uptake, while inhibiting lipolysis by blocking the activity of hormone-sensitive lipase [173].

The complex etiology of insulin resistance is not precisely defined. However, evidence suggests that elevated free fatty acids play an important role in its development [33]. When insulin fails to achieve its biological activities in the adipose tissue, free fatty acids are released into the circulatory system. These free fatty acids may be redirected to other organs with a limited lipid storage capacity, such as such as the skeletal muscle and liver [174–176]. In the skeletal muscle, free fatty acid accumulate as long-chain Acyl-CoA (LC-CoA), diacylglycerol (DAG) and ceramides which induce the activation of serine/threonine kinases. In addition, free fatty acids activate reactive oxygen species and inflammatory signaling proteins through the activation Toll-like receptor (TLR) family and indirectly through the secretion of cytokines, including TNF-α, IL-1β and IL-6 [177]. The inflammatory proteins together with the kinases phosphorylate the serine residues of IRS-1 rendering them poor substrates for insulin receptors, resulting in the inhibition of the series of events that trigger the translocation of glucose transporters and glucose transport into the cell [167,177,178].

In the liver, elevated levels of DAG due to intracellular lipid accumulation, impairs insulin signaling by activating PKC isoforms which target and phosphorylate insulin receptor substrate 2 (IRS-2), leading to decreased insulin receptor kinase activity [179]. Furthermore, decreased insulin receptor kinase ultimately leads to the increase in the translation rate of key enzymes involved in gluconeogenesis (glucose-6-phosphatase) and glycogenolysis (pyruvate carboxylase and phosphoenolpyruvate carboxykinase). Activation of these enzymes leads to an increase in hepatic glucose production and a decrease in hepatic glucose uptake. In the initial stage, β-cells increase insulin release sufficiently to overcome insulin resistance, maintaining normal blood glucose levels. However, continuous exposure of β-cells to high levels of glucose

and fat metabolites leads to β-cell failure [180]. Beta-cell destruction, together with decreased insulin sensitivity of the three target tissues (skeletal muscle, adipose tissue and the liver), ultimately lead to T2DM [175,181].
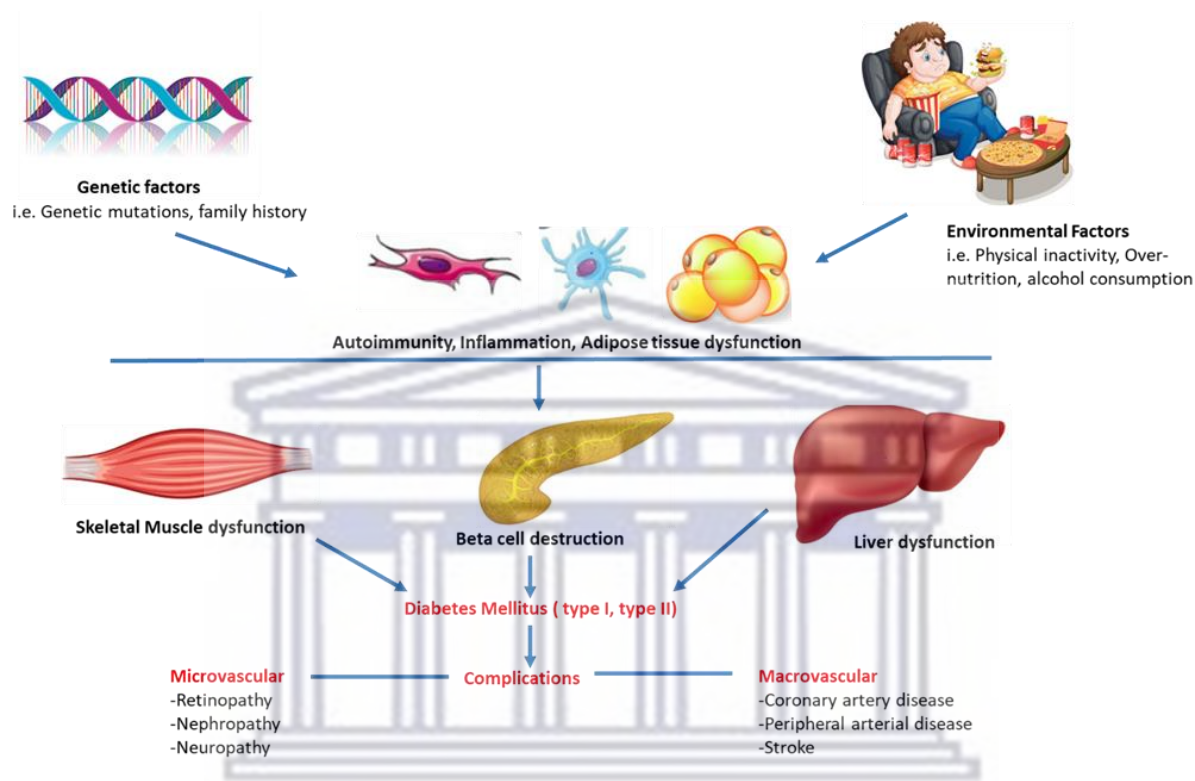
Figure 2.2: Illustration of the interplay between genetic and environmental factors in the etiology of diabetes mellitus. Figure adapted from: Al-Goblan et al. [170]; Arner [175]; Huang et al. [179]; Cerf [181]; A. Ramachandran [182] and Low Wang et al. [183].

Symptoms of T2DM are often not severe or may be absent. In the absence of routine screening, blood glucose concentrations increase sufficiently to cause pathological and functional changes, which may be present long before the diagnosis is made [182]. Type 2 diabetes often present characteristic symptoms such as thirst, polyuria, polydipsia, blurred vision, weight loss and sometimes polyphagia, often noted following elevated blood glucose concentrations (182). Acute complications of T2DM include ketoacidosis and hyperglycaemic hyperosmolar syndrome, which may present as stupor, coma and, in the absence of treatment, lead to death [183,184]. The long term relatively specific effects of diabetes include retinopathy, nephropathy, neuropathy and cardiovascular complications, including coronary heart disease,

ischemic stroke, peripheral artery disease and heart failure [184,185]. To date, cardiovascular diseases remain the principal cause of death among individuals living with DM, particularly those with T2DM [183]. Moreover, studies have shown that the incidence of heart failure and stroke is increasing among patients with T2DM, even in the absence of coronary artery diseases [184,186]. A large body of evidence supports hyperglycaemia as the primary driver for T2DM complications. However, many of the observed complications are often exacerbated by co-morbidities such as high blood pressure and hyperlipidemia [185,186]. Thus, current clinical strategies aimed at combatting DM should also address blood pressure and lipid targets [187]

2.13 Factors associated with Glycaemic Control

2.13.1 Sex

Sex refers to the biology at birth, which plays a key role in regulation of homeostasis in health and causes vulnerability to cardiometabolic risk factors, as well as manifestation, and management of T2DM [154]. Using an oral glucose tolerance test, it was shown that women have a higher rate of impaired glucose tolerance. A recent analysis conducted among T2DM patients who were treated with insulin showed that women had lower post prandial glucose levels than men after both lunch and dinner [188]. Choe et al. [189] showed women with T2DM were less likely to achieve optimal glucose control, even though they were less likely to smoke or drink alcohol. In most clinical studies, women with T2DM showed a higher burden of risk factors and co-morbidities as well as more cognitive and physical functional limitations than men [188].

Sex differences in glycaemic control arise from socio-cultural processes, such as different behaviours of women and men, exposure to specific influences of the environment, different forms of nutrition, lifestyles or stress or attitudes towards treatments [154]. In addition, sex difference in glycaemic control may be influenced by a complex interplay between genetics,

endocrine and social factors [154]. For instance, sex hormones affect health behavior that may influence physical appearance. Physical changes can have an impact on lifestyle, social roles and on mental health [190]. During menopausal transition, the decrease in oestrogen production is associated with body shape and a preferential increase of abdominal fat in women shifting to the android "visceral adiposity" [191]. Oestrogen can suppress food intake by causing a direct effect on the brain. The interaction of leptin, insulin neuropeptide Y (NPY) and ghrelin seem to play a pivotal role in this process [192]. Also, the hormone can directly affect fat storage by enhancing proliferation of preadipocytes by up-regulating alpha 2A-adrenergic receptors, thus promoting saturates fat accumulation. This might be mediated by polymorphisms in the ERα gene [193]. Visceral fat accumulation may also be a result of an overall imbalance of sex hormones. For instance, women with higher levels of androgens may increase body weight and visceral fat accumulation. This is often observed in women with polycystic ovary syndrome (PCOS) which features a state of androgen excess and hyperinsulinemia related to obesity, T2DM and higher cardiometabolic risk [194,195]. In addition to hormonal imbalances, gastric emptying and glucose absorption are also influenced by sex. A study conducted among healthy and diabetic individuals showed that, in both cases, gastric emptying was slower in women than in men [196,197]. Follow up studies reported prolonged gut glucose absorption in women, probably as a consequence of slow gastric emptying. Moreover, gastric emptying of carbohydrate containing meals has been demonstrated to positively correlate with postprandial glucose levels [154].

Women tend to be more prone to adverse effects of mental health [198]. According to health surveys, women are more likely to be physically inactive. Eating disorders, depression and anxiety are prevalent among women as compared to men [198]. Nevertheless, men who are living with diabetes seem to be coping well with their condition, showing a lower prevalence of depression and anxiety, better health-related qualities and positive wellbeing [198] . Mental

disorders adversely affect glycemic control, adherence to therapy and development of complications [199]. The presence of depression is said to double the mortality risk among DM patients [200]. Furthermore, the presence of these factors makes achieving treatment goals challenging among this sub-group. Therefore, sex-differences are worth considering among patients who are attending chronic care for T2DM.

2.13.2 Ethnicity/race

Type 2 diabetes is more prevalent among ethnic groups of African and Asian origin than the general population. Furthermore ethnic differences in diabetes care and control are documented in literature [201]. It was recently demonstrated that Afro-Caribbean and people of Asian origin were less likely to achieve treatment targets for HbA1c, blood pressure and total cholesterol in comparison to Caucasians. Furthermore, African American patients with T2DM tend to consistently exhibit worse outcomes and control (HbA1c >7.0%) when compared to other populations [202]. A recent retrospective analysis of data derived from a large national patient registry, reported that African American individuals living with T2DM were more likely to receive recommended process of care measures for their condition and significantly more likely to be receiving insulin therapy. Despite better adherence, this group was less likely to exhibit controlled glycaemia [203].

These reports suggest that ethnic-specific pathophysiological differences also play a role in glycaemic control. For instance, people of African origin exhibit upregulated or exaggerated beta-cell function in comparison to Europeans [204]. This may be a consequence of low adiponectin levels, greater sensitivity of the beta-cell towards free fatty acids or dietary factors, such as an increased fat-to-carbohydrate ratio [199,200]. It has been shown that the state of upregulated beta-cell function plays a central role in the risk of T2DM and predispose patients of African origin to premature beta-cell exhaustion [204]. Mohandas et al. [206] showed that

37

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Black African men had a lower insulin secretory responses to intravenous and oral stimulation. It was further demonstrated that lower insulin clearance, potentially driven by increased incretin responses, may act to preserve peripheral insulin concentrations. Low insulin clearance exacerbates the degree of insulin resistance among T2DM patients.

2.13.3 Age

It is widely known that the risk of T2DM increases with age [207]. However, contrasting age-related differences in glycaemic control among people with T2DM have been documented in literature. Ali et al. [208] showed that middle aged adults (40-60 years) diagnosed with T2DM were more likely to exhibit poor glycaemic control than those who were older than 65 years. Nanayakkara et al. [209] showed that younger adults (<60 years) were 1.5 times more likely to display uncontrolled T2DM. Romakin et al. [210] showed that younger adults were 2 times more likely to have uncontrolled T2DM. Although the participants who were younger than 65 years exhibited poor glycaemic control, the authors demonstrated that they had fewer co-morbid conditions, such as end stage renal disease, congestive heart failure and chronic obstructive pulmonary disease. However, this group was more likely to be obese, active smokers, have a longer duration of the disease and dyslipidemia [210]. It has been demonstrated that younger patients of T2DM have a more severe form of the disease, as result of a higher degree of insulin resistance and high blood glucose that is more resistant to current treatment [211].

 On the other hand, a number of studies have suggested that older adults (>65 years) with DM exhibit a more aggressive form of the disease as a result of co-morbidities, such as hypertension and hyperlipidemia, which makes it hard for patients to reach treatment targets [212]. Clinicians recognise this challenge and often implement stringent glycaemic targets on the elderly, thus increasing the incidence of hypoglycaemia among this sub-group [213] .

Hypoglycaemia in the elderly may be attributed to the effects of aging on the endocrine system [214]. In a healthy state, decreased levels of blood glucose trigger insulin secretion and glucagon and epinephrine release as counter-regulation. Epinephrine acts on the skeletal muscle and adipose tissue and decreases glucose clearance, while glucagon stimulates glucose production in the liver, thus maintaining adequate plasma glucose concentrations [210] Older adults with any form of DM often present with impaired glucose counter-regulation, making them prone to hypoglycaemia [214]. Lastly aging influences drug absorption and renal elimination, thus making it challenging to control the disease among this group of individuals [214]. Personalised diabetes care initiative that considers the different age groups should be implemented, in order to better manage the disease across the different age groups.

2.13.4 Alcohol

Alcohol consumption has profound effects on tissue and whole-body fuel metabolism which contribute to the burden of T2DM [216]. Despite this knowledge, there has been relatively little focus on how alcohol consumption influences glycemic control in type 2 diabetic patients. It has been reported that alcohol consumption (beer or gin) before meals resulted in 16-37% lower postprandial blood glucose as compared to isocaloric meals taken with water [217]. Among T2DM patients, 1 g of alcohol/kg body weight accompanied by a meal, slightly elevated postprandial plasma insulin and slightly lowered fasting plasma glucose levels the next morning. No hypoglycemic episodes were observed. A similar study reported no effect of alcoholic beverages on postprandial glucose, insulin or triglyceride levels, while free fatty acid levels were repressed [218]. The authors also investigated the effect of different alcohol contents on glycaemic response, where they observed a dose-dependent elevation of insulin levels [219,220]. Among patients who were undergoing oral anti-diabetic treatment, no effect on insulin, glucose and gastric inhibitory polypeptide was observed. However, a slightly

decreased in glucagon like polypeptide-1 response to a mixed meal with 40 g of alcohol beverage was observed [221].

In a long-term study, Banini et al. [222] showed that consuming wine during a meal for 28 days improved metabolic control among T2DM patients. This indicate that wine might mediate glucose metabolism. Nevertheless, some studies have reported impaired glycaemic control that was accompanied by higher HbA1c and fasting and postprandial plasma glucose values, following long term consumption of alcoholic beverages [223]. Furthermore, patients who were on glitazones and consumed alcohol demonstrated lower glucose levels. However, some studies reported opposite effects [224]. Studies conducted among Type 1 diabetic patients have established the relationship between alcohol and hypoglycaemia. These studies have shown that alcohol directly impairs the counter-regulatory hormonal response to hypoglycaemia [224]. It was further demonstrated that even the smallest amount of alcohol can worsen cognitive performance during mild hypoglycemia in patients with type 1 diabetes and impair the patient's ability to detect early hypoglycaemic symptoms [225]. Studies that evaluate the effect of alcohol on T2DM are lacking and needed. At present, there are no clear effects of alcohol consumption on glycaemic control in T2DM.

2.13.5 Physical Activity

Exercise has long been known for its ability to decrease or attenuate the progression of T2DM [226]. Although the beneficial effects of exercise are documented in relation to T2DM and all-cause mortality, T2DM patients are among the least likely population to engage in physical activity and the adherence rate is remarkably low [226]. Many patients are hindered from engaging in physical activity as a result of poor health, lack of support, lack of interest and lack of time [227]. Treatment guidelines recommend a minimum of 150 minutes of aerobic exercise per week, at 50 to 70% of their maximum heart rate, as well as resistance training three

times/week [228]. It was recently demonstrated that regular physical activity (aerobic exercise) among patients with T2DM produces a significant improvement in glycaemic control, yielding an average improvement in HbA1c between 0.4 and 0.6 [229].

Resistance exercise is a primary mode of physical activity that induces a change in muscle mass [228]. Apart from inducing a change in muscle mass, this type of training can improve glycaemic control through several mechanisms, including upregulating insulin signaling proteins and inducing GLUT 4 translocation to the cell membrane to facilitate glucose clearance from circulation during and immediately after exercise [228,230] . Among older adults, resistance training increased muscle glycogen stores, fat free mass and reduced HbA1c [230]. Results from two clinical trials, where the average age of participants was 66 years and the resistance-training regimen involved multiple exercises at high intensity, showed that individuals who engaged in this type of exercise exhibited a decrease in HbA1c (1.1–1.2%) [231]. It was further demonstrated that resistance training increased markers of insulin resistance and glycaemic control that is independent of changes in muscle mass in T2DM patients [232]. This shows that strength exercise is a viable method for producing favorable changes in body composition and also improving the insulin signaling pathway. Other exercise regimes, such as flexibility training, have been recommended for patients to reduce the incidence of injury [231]. However, there is no evidence that supports that flexibility exercise affect metabolic control or quality of life among T2DM. Exercise programs for people with any form of DM should assess for conditions that might be associated with increased cardiovascular disease. Also the patient's age, glycaemic levels and history of physical activity should be considered in order to lower the incidence of exercise-induced injuries [231].

2.13.6 Diet

The role of diet in the pathophysiology of diabetes was first described by Indians, who observed that the disease was prevalent among people who lived an affluent lifestyle and consumed fatty and sugary food in excess [233]. Since then, many studies have reported a strong association between a diet consisting of refined carbohydrates with the incidence of T2DM [234,235]. On the other hand, a low-carbohydrate diet (LCD) has been shown to be effective in improving blood glucose levels among Chinese patients with T2DM. (236). Similar effects were observed among Japanese patients who exhibited poor glycaemic control [237]. Furthermore, a high intake in fruits, particularly berries, spices and leafy vegetables was associated with a low incidence of T2DM and better glycaemic control [236]. Fruits, vegetables and spices contain large amounts of polyphenolic compounds that have been shown to have anti-hyperglycaemic properties [238,239]. For instance catechins and procyanidins can improve glucose metabolism in T2DM patients by modulating inflammation through decreasing the expression of IL-6 and MCP-1 and increasing the production of anti-inflammatory adipokine and adiponectin [240]. Furthermore, berberin may help regulate hyperglycaemia just as well as metformin in patients with T2DM and decrease insulin resistance by 45% [240].

Historically, a diet rich in proteins is not recommended for individuals with T2DM. It was believed that high proteins are converted into glucose following consumption, adversely raising blood glucose levels and having a detrimental effect on kidney function [241,242]. However, it has been shown that consumption of a high protein diet has no effect on glucose concentrations [243]. In addition, it was reported that consuming a high protein diet had no effect on kidney function and the progression of nephropathy. This remains true in patients with T2DM or impaired glucose tolerance [242]. Recent evidence supports a positive effect of a protein-rich diet in T2DM. These positive outcomes may be due to several mechanisms including enhanced glycaemic control, and satiety, increase in protein anabolism and weight

loss [149]. These positive effects were demonstrated in men and women with T2DM, where a diet high in protein and low in carbohydrates, lowered the postprandial glucose response and improved overall glucose control. The consumption of high protein products also improves insulin sensitivity in T2DM patients. It was further demonstrated that consuming at least four servings per day of low-fat dairy milk and yogurt products reduced fasting plasma insulin by 9% and improved IR by 11% [244]. Probiotics are a known constituent of yoghurt and they have been shown to reduce fasting blood glucose concentration and HbA1c levels in patients with T2DM and induces positive changes in lipid profiles [245].

2.13.7 Co-morbidities

The co-occurrence of other medical conditions in addition to diabetes is highly prevalent [246]. A little over 70% of T2DM patients have at least one chronic non-cardiovascular disease when diabetes is diagnosed. Patients with extensive co-morbidities may benefit less from intensive blood glucose control [247]. Wami et al. [248] showed that concomitant joint and respiratory disorders were significantly associated with the worsening of HbA1c levels among T2DM patients. Vitry et al. [249] showed that the presence of depression, cancer, chronic obstructive pulmonary disease, dementia and Parkinson's disease may negatively influence the therapeutic management of T2DM. Individuals with chronic illness reported a number of barriers to self-care such as physical limitations, lack of knowledge, financial constraints, logistics of obtaining care and the need for social and emotional support. The specific combination of co-morbidities in T2DM patients has been found to impact their ability to prioritise and manage the disease [250]. Patients with conditions considered unrelated to diabetes may need additional support in making decisions about care priorities and self-management activities. While the presence of diabetes "concordant" conditions i.e. sharing the same management goals) tends to be positively associated with quality of care [251,2520], certain "discordant" co-morbidities like depression and arthritis, impact on treatment options, posing barriers to

lifestyle changes and self-care behaviours recommended for diabetes management [246]. Co-morbidity can be regarded as yet another patient characteristic that needs to be accounted for when formulating individualized diabetes treatment targets. It is imperative for clinicians to achieve treatment targets of these co-morbidities whilst addressing T2DM.

2.14 Treatment for Type 2 Diabetes Mellitus

2.14.1 Metformin

Metformin is one of the most commonly prescribed anti-diabetic drugs world-wide [253] . Metformin has been used as the initial glucose lowering drug in T2DM since the early 1950s [254]. Furthermore, the drug is one of two oral anti-diabetic medications that made WHO's list of essential medications and the only approved anti-diabetic agent under the class of drugs known as biaguanide [255]. In the year 2013, metformin was prescribed to 83.6% of individuals living with diabetes in the United Kingdom. Meanwhile in the United States of America, metformin was the 8th most prescribed drug between 2008 and 2012, with the number of prescriptions increasing from 51.6 million in the year 2008 to 61,6 million in 2012 [254]. Metformin owes it popularity to a good safety profile, high effectiveness rate and low cost [255].

The most frequently reported side effects related to metformin therapy are gastrointestinal complications, with about 5% of patients demonstrating the inability to tolerate any dosage [256]. Furthermore, metformin is thought to increase the risk of rare but serious events of lactic acidosis, more especially in patients exhibiting renal insufficiency [257]. In comparison to other anti-diabetic agents, metformin is regarded as the best initial choice for improving glucose uptake in peripheral tissue without imposing the risk of hypoglycemia [254].

2.14.2 Pharmacokinetics of metformin

Metformin is not metabolized but rather excreted unchanged in the urine. About 50% of an orally administered dose is absorbed into the systemic circulation. The half-life of the drug measured in plasma is between four and eight hours in individuals without renal dysfunction, and the clearance exceeds glomerular filtration rate, consistent with tubular secretion [258]. Metformin is distributed to various parts of the body, including the intestine, liver and kidney, by organic cation transporters (OCTs) [258]. In the intestine, metformin uptake is mediated by plasma monoamine transporter (PMAT) together with organic cation transporter (OCT3), and transported into the blood stream by OCT1. Organic Cation-1 is believed to mediate hepatic metformin uptake possibly with the aid of OCT3 [258]. Furthermore, metformin is removed from target tissues by multi-antimicrobial extrusion protein 1 (MATE1) and passed from proximal tubule cells into the urine via MATE1 and MATE2 [258,259].
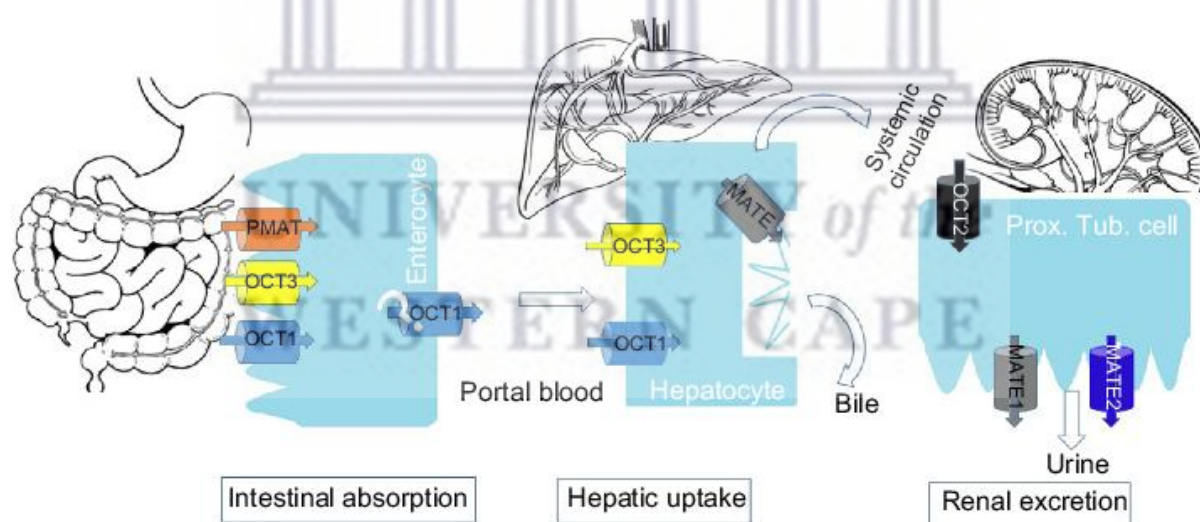
Figure 2.3: Illustration of the transport of metformin by organic cation transporters.
Source: Dawed et al. [260]. Abbreviations: MATe, multidrug and toxin extrusion antiporter; OCT, organic cation transporter; PMAT, plasma membrane monoamine transporter; Prox. Tub., proximal tubule.

2.14.3 Pharmacodynamics of Metformin

The motive behind metformin prescription is to lower blood glucose to safe levels and preventing diabetes-induced complications [261]. The mechanism of action of metformin in alleviating symptoms of diabetes remains a subject of intense research. However, it has been suggested that metformin exert its anti-diabetic properties through the inhibition of the hepatic production of glucose, reduction of intestinal glucose absorption and improvement of glucose uptake and utilization in peripheral tissues [258,262,263]. In addition, metformin may also improve insulin signaling, decrease fatty acid and triglyceride synthesis, and increase fatty acid β-oxidation [258]. This may be accomplished in part by the phosphorylation and activation of AMP-activated protein kinase (AMPK) in the liver, which in turn inhibits lipid synthesis and Glut 4 independent glucose uptake [264].

In addition, the molecular components LKB1/STK11 and ATM have been shown to play a role in the phosphorylation of AMPK following metformin administration [258]. Although the direct molecular targets remain a subject of debate, literature suggest that metformin specifically inhibits complex I of the mitochondrial respiratory chain, leading to the activation of AMPK by increasing cellular AMP: ATP ratio. Activated AMPK phosphorylates and inactivates HMG-CoA reductase, targeting rapamycin (MTOR), Acetyl-CoA carboxylase 2 (ACC-2), Acetyl-CoA carboxylase (ACC), glycerol-3-phosphate acyltransferase and carbohydrate response element-binding protein (ChREBP), thereby suppressing the expression of key lipogenic transcription factors such as sterol regulatory binding protein 1c (SREBP-1) [265].
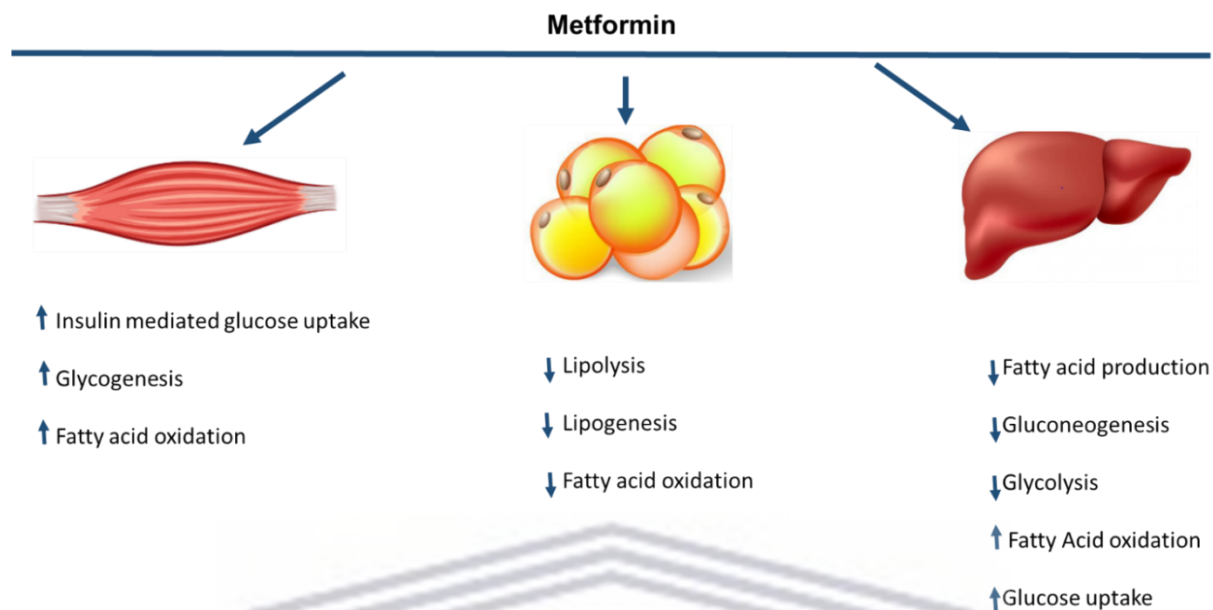
Figure 2.3: Illustration of the effect of metformin administration in the liver, skeletal muscle and adipose tissue. Figure adapted from Gong et al. [258]; Alexandre at al. [262]; Cho et al. [264] and Srivastava et al. [265] .

2.14.4 Add-on oral anti-diabetic medication –Sulfonylureas

Sulfonylureas (SUs) were originally introduced for clinical use in the 1950s and they have remained the mainstay of pharmacotherapy in the management of T2D [266]. Sulfonylureas are classified on the basis of their hierarchy of development and duration of action. In terms of development, SUs are classified into conventional and modern. Modern SUs include glimepiride, gliclazide modified release, glipizide modified release and gliclazide [267], while conventional SUs only include glibenclamide. In terms of duration of action, glipizide and gliclazide are classified as intermediate-acting. On the other hand, glibenclamide, glimepiride, gliclazide and glipizide are classified as long-acting SUs. Both classes are combined and termed second generation SUs [267].

The first generation of SUs are rarely used, however, the second generation which includes glibenclamide, glipizide, gliclazide and glimepiride are now some of the most prescribed anti-diabetic agents [266]. Second generation SUs are indicated to be used alone or in combination

with other anti-diabetic drugs including metformin [268]. Furthermore, modern SUs are initiated early in the course of T2DM, to achieve maximum glycaemic benefits and obtain the benefits of metabolic memory [266]. Although the mechanism of action of SUs is well established, their safety has been a subject of debate. Literature suggest that SUs may induce hypoglycaemia as well as weight gain among patients. Other SUs are believed to induce β-cell apoptosis, increase the risk of ischemic complications and contribute to non-fatal cardiovascular (CV) outcomes and all-cause mortality [269]. However, there is not enough evidence to support these claims. Other side effects include alcohol-induced flushing or disulfiram-like effect, cholestatic jaundice, macular erythema, rash and blood dyscrasias, dizziness, thrombocytopenia, anaemia, leukopenia, headache and increased blood pressure [266].

2.14.5 Pharmacodynamics of Sulfonylureas

Sulfonylureas are insulin secretagogues that stimulate endogenous insulin secretion by blocking adenosine triphosphate-sensitive potassium channels (KATP) in pancreatic β-cells, by binding to the sulfonylurea receptor (SUR) subunit on the plasma membrane [270]. Blocking the KATP channels inhibits the influx of K^+, consequently depolarising the membrane and facilitating influx of Ca^{2+} ions into the cell. The increase in intracellular Ca^{2+} leads to increased fusion of insulin granules with the cell membrane, thereby allowing an increase of insulin secretion and a decrease in glycaemic levels [266, 271]. Modern Sus such as glimepiride, stimulate insulin secretion by binding to a specific site on the KATP channel of pancreatic β-cells. This distinct feature of modern SUs leads to a lower inhibition of the KATP channel, hence the reduced risk of hypoglycemia in comparison to conventional SUs [267,269]. In addition, a number of extra-pancreatic properties have been defined in the literature. For instance, glipizide has been shown to reduce hepatic and metabolic clearance of insulin. This is accompanied by a decrease in glucagon secretion from pancreatic α-cells

[266,272], whilst some SUs have been shown to decrease hepatic glucose production and improve fasting glycaemia [266]. Additionally, modern SUs act as insulin sensitizers, by increasing GLUT4 translocation and glucose transport. In the adipose tissue, SUs inhibit produce lipolysis, triglyceride lipase and increase uptake and oxidation of glucose [266].
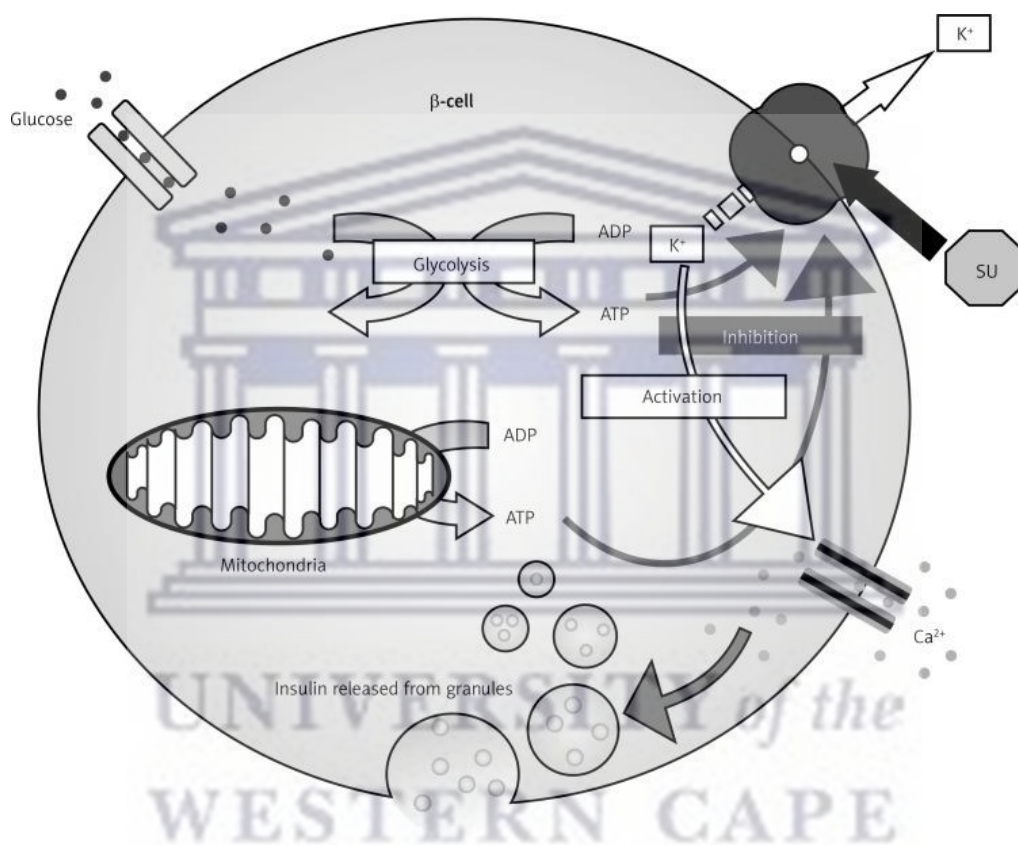
Figure 2.4: Mechanism of action of sulfonylureas on pancreatic β-cells. Source: Sola et al. [269] Abbreviations: ATP, Adenosine triphosphate; ADP, Adenosine diphosphate; SU, sulfonylureas; K^+, potassium ion.

2.14.6 Pharmacogenomic of anti-hypertensive and anti-diabetic drugs

The goal of hypertension and T2DM treatment is to maintain satisfactory metabolic control, to minimize diabetes and hypertension-related complications, and to improve the quality of life [105,273]. Despite the availability of a variety of anti-hypertensive and anti-diabetic drugs, approximately 25-50% of treated patients treated with any class of anti-hypertensive or anti-

49

diabetic drug achieve adequate control of blood pressure or blood glucose. The disappointing outcome is likely a result of non-adherence to medication as a consequence of adverse reactions, and/or interindividual variability that is brought by genetic factors [258,274,275]. It has been postulated that genetic factors influence about 30-50% of blood pressure increase [276]. In the past two decades, many studies have examined the genes associated with hypertension and T2DM. These studies reported several genetic polymorphisms, including single nucleotide polymorphisms (SNPs) that were associated with both diseases [277–279]. These studies further demonstrated that genetic polymorphisms are not only involved in the pathogenesis, but they also contribute to the large interindividual variability that is observed in anti-hypertensive and anti-diabetic treatment response [280]. This discovery has opened an opportunity for pharmacogenomic investigations and potential individualisation of drug therapy [9]. Taking into consideration the high prevalence of both diseases and the low rate of their control, the opportunity to identify the most effective drug agent for an individual patient prior to initiation of therapy may be beneficial to the patient and also minimize medical costs. Considering that the current method of prescribing medicine is predominantly empirical, and frequently involves a trial and error approach to find the optimal regimen for a given patient. The goal of hypertension and T2DM pharmacogenomics is to use a genetic-based approach that accounts for clinical and socio-demographic parameters to select the appropriate drugs with maximal efficacy and least for adverse effects.

2.15 Single nucleotide polymorphisms as predictors of anti-hypertensive drug response

2.15.1 Hydrochlorothiazide

 Nearly half of patients on hydrochlorothiazide therapy fail to reach treatment targets [281]. Thus, a number of studies have evaluated polymorphisms in candidate genes or in Genome Wide Association Studies (GWAS) as predictors of blood pressure responses to this drug [282].

The gene that encodes Alpha-adducin (ADD1) was one of the first candidate genes to be investigated with regards to the anti-hypertensive drugs [283]. Alpha-adducin is a cytoskeleton-associated protein that modulates ion transport. Carriers of the T allele ADD1 rs4961 polymorphism showed a reduced baseline plasma renin activity and better anti-hypertensive response to hydrochlorothiazide when compared to carriers of the GG genotype [284]. Similar observations were made in a study conducted among Caucasian patients with hypertension [285]. It was further demonstrated that the ADD1 rs4961 polymorphism may modulate renal sodium handling by altering ion transport across the cell membrane. Although these studies reported a positive association between ADD1 rs4961, hypertension and thiazide response, a lack of association was reported in other studies [286].

Hydrochlorothiazide induces an anti-hypertensive effect indirectly through RAAS inhibition. Therefore, some studies have also explored the association of polymorphisms in the gene encoding ACE with blood pressure response to hydrochlorothiazide [287]. In Finnish men with hypertension, the insertion/deletion (I/D) mutation of ACE was not associated with blood pressure response to hydrochlorothiazide [287]. However, Zhou et al. [288] showed that the blood pressure lowering effect of hydrochlorothiazide was greater in patients carrying the DD genotype. Moreover, a study conducted among Han Chinese patients with hypertension, who were treated with a low dose of hydrochlorothiazide, showed that the anti-hypertensive effect of the SNP was gender-specific. The authors further demonstrated that D/D and I/I genotypes were associated with a greater BP response in men and women respectively [289].

The ADRB2 and NOS3 genes are central components of RAAS [290], while the NEDD4L gene encodes a ubiquitin ligase that degrades the epithelial sodium channel, thereby affecting sodium reabsorption in the distal nephron [291]. Polymorphisms in these genes were previously associated with hypertension and blood pressure response to hydrochlorothiazide

http://etd.uwc.ac.za/

[292,293]. For instance, a study conducted among Caucasian hypertensive patients who were randomised to thiazide diuretic and beta-blocker treatments, showed that carriers of NEDD4L rs4149601 (G allele) had better anti-hypertensive response to thiazide in comparison to participants who were treated with beta-blockers with the AA genotype. Also, carriers of the G allele were protected from cardiovascular events [293]. In addition, better blood pressure response to hydrochlorothiazide was observed among Caucasian patients who were carriers of the G/C haplotype of NEDD4L rs4149601 and rs292449. These findings were not replicated among African-Americans [294]. Furthermore, a study comprised of a mixed cohort undergoing hydrochlorothiazide treatment, showed that carriers of the AA and AG genotype of ADRB2 rs2400707 had a greater reduction in whole-day ambulatory blood pressure [295], whilst carriers of the CC genotype of NOS3 rs2070744 showed an increased risk of resistance to medication in comparison to carriers of the CT and TT genotypes [296].

The WNK1 gene encodes lysine deficient protein kinase 1 (WNK1), an enzyme that regulates cation-Cl$^-$ cotransporters (CCCs), including sodium chloride cotransporter (NCC), basolateral Na-K-Cl symporter (NKCC1) and potassium chloride cotransporter (KCC1) located within the kidney. Cation-Cl$^-$ cotransporters regulate blood pressure by transporting ions in and out of the cell [297]. Owing to the function of CCCs, polymorphisms in the WNK1 genes have been associated with blood pressure disorders including hypertension [298]. A study conducted in a mixed population composed of 50% black individuals showed that the genotype CC of WNK1 rs2277869 was associated with a greater reduction in whole ambulatory blood pressure following hydrochlorothiazide treatment. Similar effects were observed among carriers of the CC genotype of WNK rs2107614 [295]. The mean decline in ambulatory blood pressure among carriers of CC was 7.2/3.6 mm Hg greater than in TT homozygotes. The authors further demonstrated that this SNP was associated with statistically significant differences in urinary potassium excretion at the end of diuretic therapy [299].

SH2B adapter protein 3 (SH2B3) is a protein that functions as a regulator of signaling pathways, relating to inflammation, recently identified as a key driver of human hypertension and renal disease [300]. On the other hand, disruptor of telomeric silencing 1-like (DOT1L) plays an important role in the methylation of histone H3 lysine 79 [301]. It was recently demonstrated that the T allele of DOT1L rs2269879 was associated with a greater blood pressure response to hydrochlorothiazide among Caucasian participants [302]. In contrast, Caucasian carriers of C allele of SH2B3 rs3184504 were more likely to exhibit reduced blood pressure in response to hydrochlorothiazide. Whereas, African American carriers of the same allele showed a slight increase in blood pressure following hydrochlorothiazide treatment [303]. These findings are yet to be replicated in other populations. Even so, it appears that polymorphisms in these genes hold a promise for personalised hydrochlorothiazide treatment.

The quest for SNPs that influence blood pressure in response to thiazide diuretics, is not limited to polymorphisms that occur in pathways that are directly associated with their mechanism of action. For instance, a number of studies have explored SNPs that occur in genes that encode proteins such as apolipoprotein A5 (APOA5), CSMD1 CUB and Sushi multiple domains 1(CSMD1), and YEATS domain containing 4 (YEATS4) [304,305]. Using data from the Genetic Epidemiology of Responses to Antihypertensive (GERA) study, Turner et al. [306] identified a region of chromosome 12q associated with the antihypertensive responses to hydrochlorothiazide among African Americans. The study demonstrated that haplotypes in this region composed of polymorphisms rs317689, rs315135, rs7297610 (YEATS4) and fibroblast growth receptor substrate 2 (FRS2) were associated with diastolic blood pressure responses to hydrochlorothiazide. Furthermore, ATT and ATC haplotypes were more frequently observed among poor responders than in good responders of hydrochlorothiazide [306]. It was further suggested that the blood pressure lowering effect of the other two SNPs may be dependent on the presence of rs7297610 (YEATS4). Similar effects were observed among Caucasians and

African-American patients undergoing hydrochlorothiazide therapy [306]. The Evaluation of Antihypertensive Responses (PEAR) study further demonstrated that haplotypes composed of polymorphisms rs317689, rs315135 and rs7297610, may be potential predictors of hydrochlorothiazide responses. The study confirmed that the SNP driving the association was rs7297610. Moreover, African American carriers of the T allele exhibited less diastolic blood pressure reduction in response to hydrochlorothiazide treatment [305]. It was further demonstrated that variation at rs7297610 is associated with differential YEATS4 expression among African Americans [305]. Whether these expression differences have a role in the diminished blood pressure response that observed with hydrochlorothiazide treatment among this group remains unknown. However, this SNP could serve as a predictor of blood pressure response for people of African origin.

2.15.2 Amlodipine

Single nucleotide polymorphisms (SNPs) that occur in genes that are directly involved in the pharmacodynamics and pharmacokinetics of amlodipine, such as the voltage-gated calcium channel α1C (*CACNA1C*) [307,308] were also examined. The *CACNA1C* gene encodes for an alpha-1 subunit of a voltage-dependent calcium channel that mediates the influx of calcium ions into the cell upon membrane depolarization [309]. This gene harbors SNPs (rs2239050, rs2238032 and rs527974) that have been implicated in the pathophysiology of hypertension. Both SNPs were recently associated with uncontrolled hypertension among Caucasian patients [308,310]. On the contrary, Japanese carriers of the promoter variant rs527974 with uncontrolled hypertension showed increased amlodipine sensitivity [310]. Since amlodipine is largely metabolised in the liver by the enzyme cytochrome P450 3A5, that is encoded by the gene Cytochrome P450 Family 3 Subfamily A Member 5 (*CYP3A5*), polymorphisms in this loci have been investigated [311]. It has been suggested that Chinese carriers of CYP3A5*3/*3, CYP3A5*3 and CYP3A5*6 polymorphisms may have increased amlodipine

metabolism, as well as increased CYP3A enzyme efficacy [312]. Moreover, it has been shown that CYP3A5 is highly polymorphic in Korean men, with a CYP3A5*3/*3 genotypes displaying lower plasma amlodipine concentration compared to t CYP3A5*1/*1 carriers, further suggesting that CYP3A5 polymorphisms may have an effect on amlodipine disposition. Among African American women, the CYP3A4 392A/G promoter variant was predictive of BP response following amlodipine treatment. It was also shown that carriers of the A allele were three times more likely to reach a mean arterial pressure (MAP) of 107 mmHg [310]. Nonetheless, conflicting data has been presented regarding observed individual variability to amlodipine and polymorphisms associated with these genes. More data is required to validate the direction of association of the SNPs that occur in this gene.

Nitric oxide synthase-1-adaptor protein (*NOS1AP*) is part of the sympathetic and para-sympathetic nervous systems and is known to be involved in the pathophysiology of hypertension [313, 314]. The variant allele G of rs10494366 of the *NOS1AP* gene was associated with an increase in cardiovascular mortality among Caucasian users of amlodipine [314]. Furthermore, polymorphism rs4291 of the *ACE* gene was strongly associated with elevated blood pressure [315]. Similarly, African American carriers of the minor allele of rs11122576 of *AGT* gene (encodes angiotensinogen) who were undergoing amlodipine therapy showed a decreased risk of coronary heart diseases [316]. At the same time, patients with the AA genotype of rs1042713 (*ADRB2*) demonstrate poor efficacy of cardiovascular drugs including ACE-inhibitors [317]. Hypertensive carriers of the AA genotype of ACE rs4291 may have decreased fasting glucose levels when treated with amlodipine [318]. Nevertheless, no direct association with blood pressure response to amlodipine has been established for these SNPs.

2.15.3 Enalapril

The pharmacogenomic relevance of RAAS-associated genes with enalapril treatment response has been explored by many studies. The polymorphisms rs699947, rs1570360 and rs2010963 polymorphism is found on the Vascular Endothelial Growth Factor A (VEGFA) gene, which encodes the VEGF protein. It was previously demonstrated that the A allele of the SNP is associated with an earlier onset of hypertension in preeclampsia [319]. Indeed, lower VEGF levels were found in hypertensive patients compared with normotensive subjects [133]. Using a mixed Brazilian cohort, Oliveira-Paula et al. (2015) showed that carriers of the CA and AA genotypes of VEGFA rs699947 showed an increased response to enalapril. Although the genotypes of rs1570360 and rs2010963 showed no effect on blood pressure response to enalapril, the authors demonstrated that the AGG haplotype of the three polymorphisms was associated with a more intense blood pressure decrease in response to enalapril [133]. The mechanism in which rs699947 predispose patients to hypertension and also enhance enalapril treatment is not known. Additionally, NOS3 rs2070744 is a known predictor of hydrochlorothiazide treatment outcome. Among enalapril treated patients, CC genotype of NOS3 rs2070744 was associated with a greater reduction in blood pressure (136). whilst, GG and CG of ADRB2 rs1042714 were associated with increased enalapril sensitivity among Europeans with left ventricular hypertrophy [320]. The direct effect of this SNP with regards to blood pressure in response to enalapril is yet to be investigated.

Polymorphisms rs1799722 sits on the promoter region of Bradykinin receptor B2 (BDKRB2), a gene that encodes the BDKRB2 protein. The CT and CC genotypes of BDKRB2 rs1799722 showed an increased response to enalapril treatment among Brazilian patients with mild-moderate hypertension [136]. The T allele of the same SNP was associated with persistent cough among patients who were treated with ACE inhibitors [321]. Polymorphisms such as rs495828 and rs2306283 that occur in genes that encode histo-blood group ABO system

transferase (ABO) and organic anion transporting polypeptide 1B1 (SLCO1B1) were also associated with enalapril induced cough in different racial groups [322].

2.16 Single nucleotide polymorphisms as predictors of anti-diabetic drug response

2.16.1 Metformin

Metformin is not metabolised in the body, but very efficiently excreted in the urine. Therefore, the glucose lowering effect of metformin is not affected by genetic variation in metabolizing enzymes [260]. Also, metformin cannot diffuse through membranes passively but it is dependent on drug transporters for the absorption, distribution and elimination [260]. Genetic variation in the SLC22A1 gene encoding the OCT1 transporter and the glucose lowering effect of metformin both in animal model and human subjects are defined in literature. It was shown that South Indian carriers of two copies of the variant allele of SLC22A1 rs622342 (AA) had a greater chance of responding to metformin therapy [280]. Wu et al. [323] showed that carriers of the AA genotype of SLC22A1 rs622342 had high fasting plasma glucose, HbA1c and HOMA-IR as compared to carriers of the C allele. The authors further demonstrated that the association of HOMA-IR with rs622342 was gender dependent. The data presented in this study showed that rs622342 could be a predictor of insulin sensitivity in patients with T2DM treated with metformin [323]. The same SNP was associated with reduced uptake activity of metformin among African and European individuals. On the other hand, the del/del genotype of SLC22A1 rs36056065 was associated with the risk for metformin-induced gastrointestinal side effects among T2DM patients. Similar effects were also observed among carriers of SLC22A1 rs12208357 and rs72552763 [324]. In addition to metformin-induced adverse reactions, SLC22A1 rs72552763 was associated with a significantly higher renal clearance of metformin [325]. The variants rs316019 and rs596881 occur in the 3' untranslated region of SLC22A2 and they are frequently detected in almost all ethnicities [325]. Both polymorphisms

showed significantly lower activity in metformin transport in healthy Korean and Chinese subjects, resulting in lower renal clearance of the drug [325,326]. Among Africa American individuals, the minor allele of rs316019 was associated with a lower disease progression [327]. However, a study conducted among health individuals (African American and Caucasian) showed that renal clearance and the net secretion of metformin were significantly higher in the variant genotype of rs316019 than in the ancestral allele [325]. A meta-analysis study composed of 5434 patients with T2DM, reported that there was no significant association of the variant allele with glycaemic response [324] .

Specificity protein 1 is a transcription factor that is encoded by Sp1. Specificity protein 1 is involved in the regulation of SLC membrane transporters [328]. The C allele of Sp1 rs2683511 was associated with decreased HbA1c following metformin treatment in people with T2DM. Also, the C allele of Sp1 rs10747673 was associated with decreased metformin clearance following metformin treatment, leading to reduced exposure and a corresponding increase in HbA1c levels, which is indicative of decreased metformin efficacy [329]. The variants rs784888 was also associated with metformin response in T2DM. It was recently demonstrated that carrier of the CC genotype of rs784888 with T2DM may exhibit increased clearance of metformin leading to worse response to the drug [329].

Calpain-10 (CAPN10) is located at 2q37 and encodes a ubiquitously expressed member of the calpain cysteine protease family. Fine mapping and positional cloning suggested that the calpain-10 (CAPN10) gene might serve as an important T2DM susceptibility gene [330]. Carboxypeptidase A6 (CPA6) encodes a metallocarboxypeptidase enzyme which catalyses the release of C-terminal amino acid, and has functions ranging from digestion of food to selective biosynthesis of neuroendocrine peptides [331]. On the other hand, pre-mRNA processing factor 31 homolog PRP31 is a protein that forms part of the spliceosome complex encoded by

the PRPF31 gene. Common variants in PRPF31 and CPA6 were associated with metformin response in T2DM patients [332]. European carriers of the CC and CG genotype of PRPF31 rs254271 may have decreased response to metformin when compared with GG carriers who demonstrated an increase in metformin response. Similarly, patients with the CC and CT genotype of CPA6 rs2162145 may have decreased response to metformin as compared to carriers of the TT genotype [332]. On the other hand, carriers of the AA genotype of rs3792269 (CAPN10) may have an increased response to metformin. However, patients with the GG genotype may have decreased metformin response [333]. Other polymorphisms associated with metformin response include PPARGC1A (rs10213440), PCK1 (rs4810083), ABCC8 (rs4148609), ITLN2 (rs6701920) , GCG (rs6733736) and FMO5 (7541245) [334].

2.16.2 Sulfonylureas

Sulfonylureas are prescribed as second-line therapy during the progression of disease or failure to achieve satisfactory effects with metformin alone [335]. Substantial interindividual differences are shown in the glucose-lowering effect of sulfonylureas. Cyclin-dependent kinase 5 (CDK5) regulatory subunit- associated protein 1-like (CDKAL1) is one of the genes whose strong association with T2DM has been reported in several GWA studies [336]. The G allele of CDKAL1 rs7754840 was associated with T2DM among a Korean population. Furthermore, the GG genotype of CDKAL1 rs7754840 was significantly associated with higher risk of resistance to sulfonylureas treatment in an Iranian population [336].

ATP-binding cassette transporter sub-family C member 8 (ABCC8) is a gene that provides instructions for making the sulfonylurea receptor 1 (SUR1) protein [337], while Potassium Inwardly Rectifying Channel Subfamily J Member 11 (KCNJ11) encode Kir6.2 subunit of the ATP-sensitive potassium KATP channel [338]. Both genes are adjacent to one another on human chromosome 11p15.1 and play an important role in the SU-stimulated insulin release

[339]. On the other hand, the SCNN1B gene encodes for the β subunit of the epithelial sodium channel ENaC and is associated with variable response to SUs. *In silico* analysis showed that ABCC8 (rs757110, rs1799854, rs1799859) and KCNJ11 (rs5219) polymorphisms were associated with glycaemic response to SUs [339]. Carriers of the rs757110 (G allele) treated with gliclazide exhibited decreased HbA1c than carriers of homozygous TT [340]. Similar effects were observed among Chinese individuals who were treated with hydrochlorothiazide. These findings indicate that G allele carriers were hypersensitive to SUs. Although carriers of the G allele exhibited increased SU sensitivity, the variant was not associated with the risk of hypoglycaemia [341]. In contrast, Caucasian carriers of the CT genotype of rs1799854 had a higher HbA1c SU treatment. Opposite effects were reported for rs1799859, where carriers of the AA genotype showed lower HbA1c levels compared with GG and GA genotype carriers [342]. In addition, a large Italian cohort study showed that carriers of rs5219 had a relative risk for secondary SU failure and hypoglycaemia [341,343]. while in a Slovakian population, the presence rs5219 and rs5215 was not associated with hypoglycaemia [341]. In another study, the T allele of KCNJ11 rs5219 was associated with decreased KCNJ11 expression in glibenclamide treated Caucasian patients with T2DM [343]. It was further demonstrated that rs5219 was a predictor of SU failure among Asians [341]. In addition, the UTR variant of KCNJ11 (rs5210) was associated with a positive gliclazide response among Chinese patients [341]. Furthermore, Colin et al. [344] showed that the variant allele of SCNN1B rs889299 was associated with oedema in T2DM patients that were treated with glibenclamide. This effect was predominantly observed among Caucasian carriers of the A allele.

Cytochrome P450 2C9 (CYP2C9) is a hepatic enzyme that is involved in the metabolism of 10%–20% of currently used drugs and has a major role in the metabolism of sulfonylureas [345]. Many SNPs in the gene (CYP2C9) that encode this enzyme are associated with decreased enzymatic activity. For instance, CYP2C9*2 (rs1799853) and CYP2C9*3

(rs1057910) in exons 3 and 7 respectively, both of which are associated with impaired function and poor metabolism phenotypes [346]. It was recently demonstrated that T2DM patients with the CC genotype of CYP2C9*2 may be less likely to achieve a HbA1c level of <7% as compared to patients with the TT genotype following treatment with SUs. Whereas the CC genotype of CYP2C9*3 was associated with increased response to SUs. In the largest study to date on the effect of CYP2C9 variants on therapeutic response to SUs, the authors showed an association of CYP2C9*2 and CYP2C9*3 alleles with greater glycaemic response to SUs and a lower rate of treatment failure [347]. In contrast, elderly patients who carry the two polymorphisms showed an increased risk of hypoglycaemia [348]. Both polymorphisms lead to reduced enzyme activity, increased plasma drug concentrations and enhanced activity of the drug. This effect can either be beneficial to the patient or induce hypoglycaemia [348].

The effect of SNPs on glycaemic response following treatment with metformin and SU combination therapy is not well documented in literature. In Lebanese patients receiving metformin and SU combination therapy, who were carriers of the AA (SLC22A1) and AC (CYP2C9*3) *1*3 genotypes benefited the most following a six-month treatment. These findings have confirmed the role of CYP2C9 in the pharmacokinetics of SUs [345]. Polymorphisms ABCC8 rs757110 showed no association among Egyptian patients on metformin and glimepiride combination therapy [349]. More studies on the effect of metformin and SU combination therapy are needed in order to better stratify patients and individualise T2DM dual therapy.

2.17 Epistatic interactions in drug response phenotypes

The two common approaches that are used in pharmacogenomics studies are candidate gene and GWAS [350,351]. These methods examine common genetic variations across the entire human genome by utilizing high throughput genotyping technologies to assay hundreds of

thousands of SNPs [350,351]. In the last decade, multiple GWAS and candidate gene studies have been conducted in an attempt to identify common variants that are associated with ant-diabetic and antihypertensive treatment response across different populations [351,352]. Since a large portion of the genetic predisposition to drug response phenotypes seems to be hidden in multigenic and multifactorial complex traits, these approaches may overlook the association that can be detected when combination of multiple genomic regions are assessed [353]. To assess this phenomenon, novel approaches that consider the interactions among polymorphisms from different genes within drug response pathways have been employed in pharmacogenomic studies. One of these approaches involves the use of the multifactor dimensionality reduction (MDR), a software that measures high-order gene-gene interactions with the use of relatively small sample sizes. This method is non-parametric and it does not assume any genetic model, it is best suited for gene-gene interaction of complex diseases whose mode of genetic inheritance is unknown [354].

Using MRD, Silva et al. [136] reported a significant interaction between NOS3 and BDKRB2. The authors further demonstrated that this interaction was implicated in enalapril treatment response among Brazilian patients with non-complicated hypertension. An interaction between these genes is expected, since both resulting proteins are involved in the ACE signaling pathway [136]. Although single locus showed that the CC genotype of BDKRB2 rs1799722 was not associated with a positive response to enalapril, gene-gene interaction analysis showed that the CC genotype for this polymorphism combined with the TT genotype for rs2070744 NOS3 polymorphism was more frequent in poor responders [136]. While the combination of BDKRB2 CC genotype with NOS3 TC genotype was more frequent among good responders [136]. Recently, Oliveira-Paula et al. [355] showed that the combination of BDKRB2 and NOS3 are associated with the anti-hypertensive effect of enalapril only in the presence of the GG genotype of PRKCA rs16960228. These findings are underestimated when single

BDKRB2 genotypes alone are analyzed, thus highlighting the relevance of gene-gene interaction analysis.

The relevance of gene-gene interactions was also assessed in anti-diabetic drug response. Becker et al. [356] reported a significant interaction between MATE1 rs2289669 and SLC22A1 rs622342 among Caucasian patients. Furthermore, patients with the genotype combination rs622342 AA and MATE1 rs2289669 AA showed a greater reduction in HbA1c levels, whereas patients with the SLC22A1 rs622342 CC and MATE1 rs2289669 GG genotype exhibited increased HbA1c levels [356]. Both SNPs are located in the intronic region of their respective genes. Therefore, it is possible that the glycaemic lowering effects of these polymorphisms is also a result of an interaction with other SNPs that are in linkage disequilibrium. An interaction between SLC22A1 rs594709 and SLC47A1 rs2289669 among Chinese individuals undergoing metformin treatment was assessed by Xiao et al. [357]. The study found a significant association between the two SNPs, which improved insulin resistance and blood lipids in response to metformin therapy. It was further demonstrated that carriers of the AA genotypes of both SNPs showed a greater decrease in fasting plasma glucose and HOMA-IR [357]. On the other hand, Naja et al. [345] showed that there was no interaction between SLC22A1 rs622342 and polymorphisms of CYP2C9 among Lebanese patients on combination therapy of metformin and SUs. These finding have highlighted the complex interactions that exist with regards to drug response across different populations. However, the effects on glycaemic response to metformin and SU combination therapy are yet to be investigated in the ethnically diverse population of South Africa.

2.18 References

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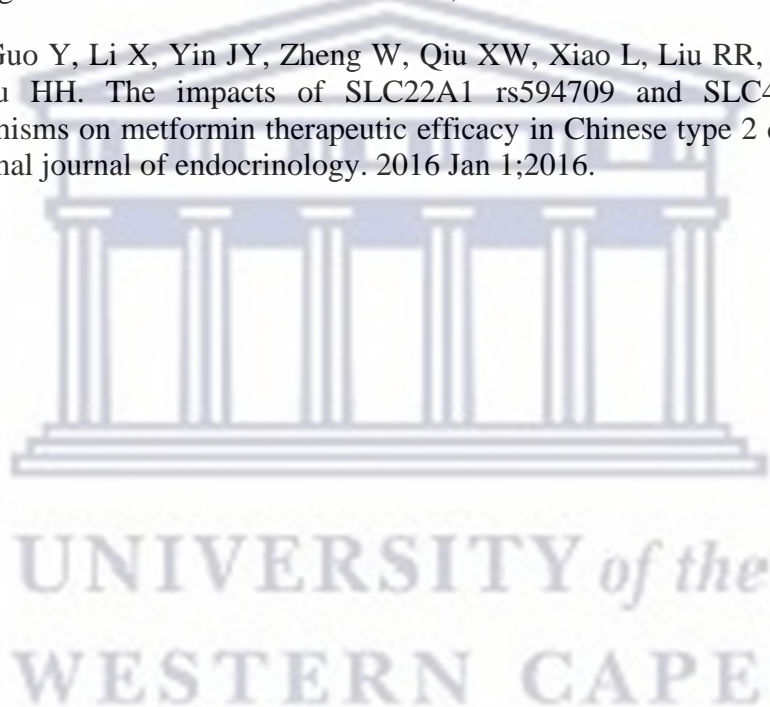
Chapter 3

Prevalence and determinants of uncontrolled Hypertension among South African adult residents of Mkhondo municipality

3.1 Introduction

The manuscript presented in this chapter examined the prevalence and determinants of uncontrolled hypertension among the residents of Mkhondo Municipality. Achieving blood pressure treatment target in individuals with hypertension is a serious public health challenge. Furthermore, the actual burden of uncontrolled hypertension is not fully understood especially in the developing countries. Such information is relevant in establishing clinical and context-specific interventions that are aimed at improving patient care in order to control the disease.

3.2 Publication details

Title	Cross-sectional study of prevalence and determinants of uncontrolled Hypertension among South African adult residents of Mkhondo municipality
Authors	CM Masilela, B Pearce, JJ Ongole, OV Adeniyi and M Benjeddou
Contribution	CM, BP, JJO and MB conceptualised, designed and implemented the study protocol. CM and OVA analysed the data and drafted the manuscript. All authors revised and approved the final draft of the manuscript for submission
Ethics	BM/16/5/19
Journal	BMC Public Health
Status	Published (https://doi.org/10.1186/s12889-020-09174-7)

3.3 Journal information

BMC Public Health is an open access, peer-reviewed journal that considers articles on the epidemiology of disease and the understanding of all aspects of public health. The journal has a special focus on the social determinants of health, the environmental, behavioural, and

occupational correlates of health and disease, and the impact of health policies, practices and interventions on the community.

3.4 Manuscript: Cross-sectional study of prevalence and determinants of uncontrolled Hypertension among South African adult residents of Mkhondo municipality

Abstract

Background: Achieving the blood pressure treatment target in individuals with hypertension is a serious global health challenge. Furthermore, the actual burden of uncontrolled hypertension is poorly understood especially in the developing countries. Therefore, this study comprehensively examined the prevalence and factors associated with uncontrolled hypertension in individuals receiving care at the primary healthcare facilities in the rural areas of Mkhondo Municipality in the Mpumalanga Province, South Africa.

Methods: In this cross-sectional study, 329 individuals attending care for hypertension were recruited from January 2019 to June 2019 at three primary healthcare centres, namely, Piet Retief hospital, Mkhondo town clinic and Thandukukhanya community health centre. Uncontrolled hypertension was defined as systolic blood pressure ≥ 140mmHg and/or diastolic blood pressure ≥ 90mmHg in accordance with the South African Hypertension Society guideline (2014). Multiple logistic regression (Forward LR method) analysis was used to identify the significant determinants of uncontrolled hypertension.

Results: The majority of the participants were 55 years old and above (69.0%), Zulus (81.2%), non-smokers (84.19%) and had been diagnosed with hypertension for more than a year prior to the study (72.64%). The overall prevalence of uncontrolled hypertension was 56.83%

(n=187) with no significant difference between sexes, 57.38% males versus 56.88% females, respectively. In the multiple logistic regression model analysis after adjusting for confounding variables, obesity (AOR=2.90; 95% CI 1.66-5.05), physical activity (AOR=4.79; 95% CI 2.15-10.65) and HDL-C (AOR=5.66; 95% CI 3.33-9.60) were the significant and independent determinants of uncontrolled hypertension in the cohort.

Conclusion: The high prevalence of uncontrolled hypertension in the study setting, can be largely attributed to obesity, physical activity and dyslipidaemia. Treatment will require the collaborative efforts of individuals, clinicians and health authorities. All these determinants should be addressed decisively so as to achieve the treatment blood pressure targets in the study population.

Keywords: Uncontrolled Hypertension; Mkhondo Municipality; South Africa

Introduction

The prevalence of non-communicable diseases (NCDs) is increasing at an alarming rate world-wide, and it has been driven by the rise in the incidence of cardiovascular risk factors such as obesity, diabetes and hypertension [1]. Hypertension is one of the important risk factors for morbidity and mortality, affecting about 1 billion people world-wide [2]. In the year 2000, 972 million people were living with hypertension and a large burden was borne by economically disadvantage countries [2], where awareness and treatment often falls short. According to the world health organization (WHO), 27.4% of men and 26.1% women have hypertension [3], though an overall prevalence of up to 54% has been reported in urban areas [4]. In the absence of effective intervention strategies to control this epidemic, the prevalence of hypertension is likely to increase in the next decade [2,4].

Hypertension is a multifactorial and highly complex disease that is characterized by a systolic blood pressure ≥ 140 mmHg and/ or diastolic blood pressure ≥ 90 mmHg [5]. The risk factors of hypertension include excessive salt intake [6], alcohol consumption and lack of physical activity [7]. Evidence suggests that hypertension has no obvious symptoms and often remains undiagnosed for a long period [8]. Furthermore, the asymptomatic and persistent nature of the disease presents a major challenge of identifying people with elevated blood pressure and providing optimal medical care [8]. When diagnosed early, lifestyle changes and pharmacological interventions are essential for the management and control of the disease [8,9]. However, poor adherence to non-pharmacological and pharmacological management of hypertension represents a serious challenge for public health in many countries [10,11].

Uncontrolled hypertension, defined as blood systolic blood pressure ≥ 140 and / or diastolic blood pressure ≥90 mmHg, has been associated with aging, clinical inertia, unemployed status and nutritional transitions [12]. Furthermore, only 25% of individuals undergoing anti-hypertensive treatment appear to be well controlled [9], while the majority of studies conducted in Africa have shown that less than a third of patients reach their treatment goals [13]. In South Africa, the prevalence of uncontrolled hypertension has been estimated at between 13.5 - 75.5% [12,14], whilst figures ranging between 19.0 – 56.0% have been reported for hypertension control [3]. On the other hand, hypertension control among individuals residing in high-income countries has been reported to be as high as 82.0% [3,15], whereas a number as low as 28.4% has also been reported in high-income countries [16]. Those who fail to reach therapeutic targets continue to be at a higher risk for cardiovascular events, kidney diseases [9,17], stroke [18], metabolic syndrome, hypertensive retinopathy [19] and dementia [20]. In addition to adherence, the multifactorial nature of hypertension presents the greatest challenge for its treatment and control [21]. Even so, only a few studies have assessed the prevalence of uncontrolled hypertension and its determining factors in South Africa, particularly within

economically disadvantaged populations. As a result, the actual burden of uncontrolled hypertension in such communities is poorly understood and awareness as well as treatment to achieve control remains suboptimal. Therefore, epidemiological data from these communities are essential in planning interventions based on local influential factors associated with the disease. On these grounds, the current study aims to comprehensively assess the prevalence, the socio-demographic and clinical determinants of uncontrolled hypertension among patients receiving health care in three public sector facilities serving the rural areas of Mkhondo municipality in the Mpumalanga province, South Africa.

Methods

Study design and settings

This was a community based cross-sectional study conducted in the Piet Retief Hospital, Mkhondo town clinic and Thandukukhanya Community Health Center in Mkhondo Municipality of Mpumalanga Province, South Africa, from January 2019 to June 2019. These health care facilities provide chronic care services for the residents of Mkhondo Municipality. Mkhondo is located in the Gert Sibande district of the Mpumalanga province and is a resource constrained community made up of a small town surrounded by farms and two townships (Ethandukukhanya and AJAX) with a population of approximately 189,036 [22].

Study population and sample size estimation

The study included individuals who were aged ≥18 years, had been diagnosed and initiated on treatment for hypertension for more than a year prior to the study. Individuals who were bed-ridden, mentally compromised, pregnant, and unable to give consent were excluded from the study.

A sample size of 334 was estimated by using the formula for cross sectional study:

$$\{N = (Z_{1-\alpha})^2 \text{ x } P (1 - P) / D^2\}$$

Given that the prevalence of uncontrolled hypertension ranged widely from 13.5 – 75.5% in South Africa [12,14], P (P = proportion with uncontrolled hypertension) of 68% was chosen for the sample size estimation. $Z_{1-\alpha}$ = 1.96 and D = absolute precision and is taken as 0.05. Five participants dropped off from the study and a total of 329 patients were included in the final analysis.

Data collection

Eligible participants were recruited sequentially at the study settings over the study period. Trained research assistants conducted face to face interviews with consenting participants by using standardized questionnaire. The questionnaire comprises socio-demographic characteristics of age, sex, level of education and employment status, lifestyle behaviours; physical activity and dietary patterns, cigarette smoking status and alcohol use, plus questions about family history of hypertension. The following clinical data were obtained from the medical records: duration of hypertension, number of anti-hypertensive drugs and drug combinations. Drug combinations included the following: Thiazide diuretic only, combination therapy (Thiazide + calcium channel blocker + angiotensin Converting enzyme inhibitor and Thiazide + calcium channel blocker + Angiotensin converting enzyme inhibitor + beta-blocker) and other (Loop diuretic monotherapy, loop diuretic + calcium channel blocker and/ or beta-blocker + calcium channel blocker + angiotensin converting enzyme inhibitor).

The level of education was categorized as no education, primary (grade 1 – 6), secondary (7 – 12) and tertiary. Employment status was categorized as unemployed, employed or receiving social grants from the government. Smoking status were categorized as never smoked or ever smoked; while alcohol use was also categorized similarly. Physical activity was categorized as active (if engaging in vigorous intensity exercise leading to increase in heart and respiratory

rate such as gardening or inactive (not engaging in any physical activity). Also, participants reported their average consumption of fruits and vegetables, as well as fast food and salt intake.

A trained research nurse conducted anthropometric measurements of weight to the nearest 0.1 kg using a digital scale (Tanita-HD 309, Creative Health Products, MI, USA) and height to the nearest of 0.1 cm using a mounted stadiometer. All measurements were taken with the participants wearing minimal clothing and no shoes. Body Mass Index (BMI) for each patient was calculated as weight (kg) divided by height in meters squared (m^2) and was categorized based on WHO criteria as obese (30 or greater kg/ m^2) or not [23].

Blood pressure (BP) was measured using a validated automated digital blood pressure monitor (Macrolife BP A 100 Plus model) according to standard protocols. The BP was recorded in triplicate and the average was used for analysis. Patients with systolic BP (SBP) of ≥ 140 mmHg and/ or diastolic BP (DBP) ≥ 90 mmHg were defined as uncontrolled BP [24].

Laboratory assessment

After an eight hour fast, the research nurse drew five millilitres of venous blood sample from each participant for laboratory assessment. The lipid profile which includes: total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL-C) and high-density lipoprotein (HDL-C) for each participant was categorized according to the guidelines of The Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA, 2017). All laboratory assays were conducted by the National Health Laboratory Services (NHLS) of Piet Retief and Ermelo Provincial hospitals in accordance with standard protocols.

Statistical analysis

Data analysis was conducted using IBM SPSS Statistics for windows, Version 25.0 (IBM Corp., Armonk, New York, USA). General characteristics of the participants were expressed

as mean ± standard deviation for continuous variables. Categorical variables were reported as frequency (percentage). The associations between socio-demographic, clinical factors and uncontrolled hypertension were examined by using bivariate analysis. Multiple logistic regression odds ratios and their 95% confidence intervals, using crude and adjusted logistic regression model analysis, helped identify the independent determinants of uncontrolled hypertension. A p-value of less than 0.05 was considered statistically significant.

Results

Of the total number of participants (n=329), 61 were males (18.5%) and 268 were females (81.5%). The majority of the participants were aged 55 years and above (69.0%), Zulus (81.2%), non-smokers (84.19%), non-alcohol drinkers (77.81%), consuming fruit and vegetables (97.87%) and fast food about three times per week (61.09%), which differed according to sex. A sedentary lifestyle was reported by 231 participants (70.21%); 22 were males and 209 were females. Consumption of excessive amounts of salt was reported by 42 participants (12.78%) of whom a higher proportion were women (n=37) in comparison with men (n=5). The majority of the participants had been diagnosed within the previous five years (n=234; 72.64%) and they were predominantly women (n=194). Table 1 provides detailed descriptive characteristics of the participants

Table 1: Demographic characteristics of the study participants disaggregated by hypertension control

Variables	All Participants (n; %)	Controlled Hypertension (n; %)	Uncontrolled Hypertension (n; %)	p-value
All	329(100%)	142(43.16%)	187(56.83%)	
Gender				0.925
Male	61(18.54)	26(18.31)	35(18.72)	
Female	268(81.46)	116(81.69)	152(81.28)	
Age (Years)				0.467
18 – 25	08(2.43)	03(2.11)	05(2.67)	
26 – 35	13(3.95)	05(3.52)	08(4.28)	
36 – 45	29(8.81)	08(5.63)	21(11.22)	
46 – 55	69(20.97)	32(22.54)	37(19.78)	
56 – 65	97(29.48)	47(33.09)	50(26.73)	
≥66	113(34.35)	47(33.09)	66(35.29)	
Ethnicity				0.813
Zulu	267(81.16)	114(80.28)	153(81.81)	
Swati	54(16.41)	24(16.90)	30(16.04)	
Not specified	08(2.43)	4(2.82)	4(2.14)	
Employment status				0.201
Employed	79(24.01)	39(27.46)	40(21.39)	
Unemployed	104(31.61)	38(26.76)	66(35.29)	
Social grant recipient	146(44.38)	65(45.80)	81(43.32)	
Educational Level				0.157
Tertiary	07(2.13)	05(3.52)	02(1.07)	
Secondary	109(33.13)	46(32.39)	63(33.68)	
Primary	140(42.55)	54(38.03)	86(45.98)	
Illiterate	73(22.19)	37(26.05)	36(19.26)	
Smoking status				0.634

Never Smoked	277(84.19)	118(83.09)	159(85.03)	
Ever Smoked	52(15.81)	24(16.91)	28(14.97)	
Alcohol consumption				0.922
Never Drank	254(77.20)	110(77.46)	144(77.01)	
Occasional	75(22.80)	32(22.54)	43(22.99)	
Fruit and Vegetable Consumption				0.987
1-3 times/week	322(97.87)	139(97.89)	183(97.86)	
Never	07(2.13)	03(2.11)	04(2.14)	
Fast Food Consumption				0.459
Never	128(38.91)	52(36.62)	76(40.64)	
1-3 times/week	201(61.09)	90(63.38)	111(59.36)	
Salt intake				0.771
Low-Moderate	287(87.23)	123(86.62)	164(87.70)	
Increased	42(12.77)	19(13.38)	23(12.30)	
Duration of Diagnosis				0.140
<5 years	95(28.88)	35(24.65)	60(32.09)	
≥5 years	234(71.12)	107(75.35)	127(67.91)	

Prevalence of uncontrolled hypertension

All the participants (N=329) had been on anti-hypertensive treatment for at least a year. The majority of the participants were on at least two or more anti-hypertensive drugs (n=260; 79.03%). Thiazide diuretic (for example hydrochlorothiazide) was the preferred drug class either alone (n=44; 13.37%) or in combination with other drugs (calcium channel blockers, Beta-blockers and Angiotensin-converting enzyme inhibitors) (86.62%). Of the 329 participants, successful treatment to the blood pressure target of 140/90mmHg occurred in 142 patients (43.62%). The overall prevalence of uncontrolled hypertension was 56.83% (n=187) with no significant difference between sexes; 57.38% males versus 56.88% females, respectively (Fig. 1).
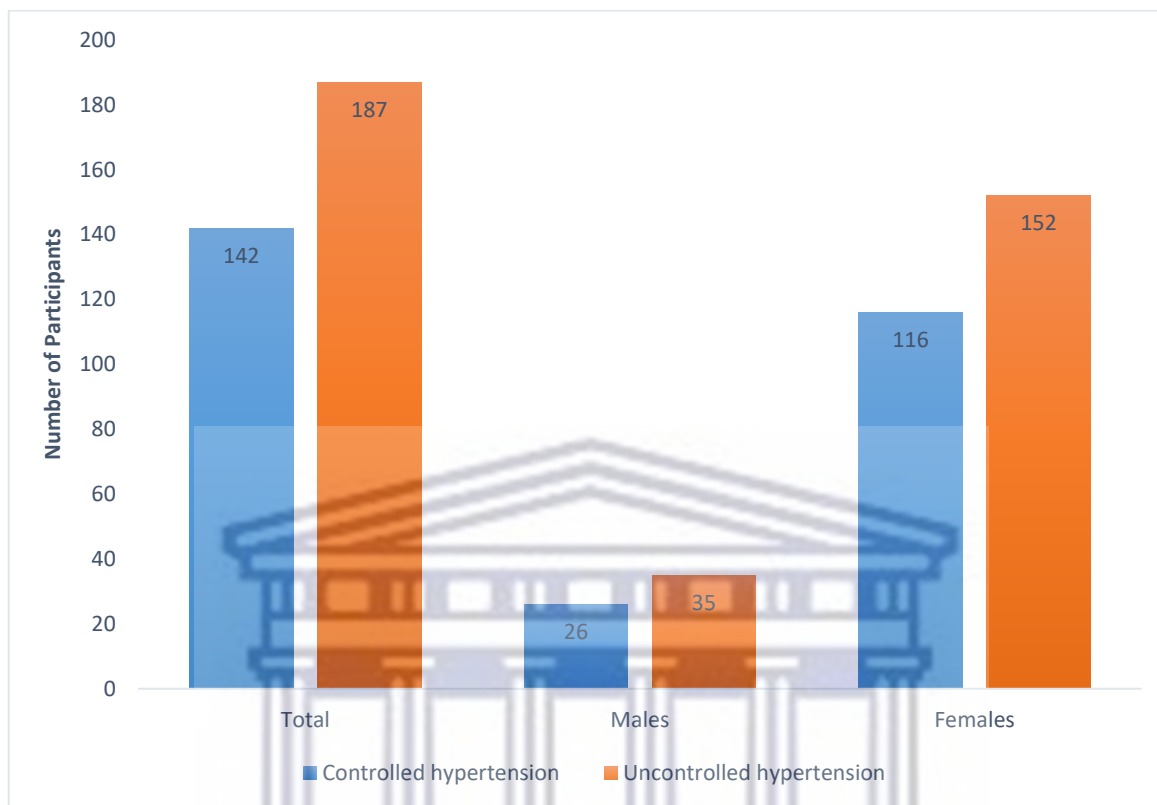
Figure 1: Prevalence of Uncontrolled hypertension

Factors associated with uncontrolled hypertension

In the bivariate logistic regression analysis (Table 2), physical activity (p<0.001), positive family history (p=0.034), low HDL-C (p<0.001) and obesity (p=0.001) were significantly associated with uncontrolled hypertension. Other risk factors such as age, gender, smoking status, alcohol consumption, Total cholesterol, LDL-C and triglycerides were not significantly associated with uncontrolled hypertension (p>0.05).

Table 2: Bivariate analysis showing the factors associated with uncontrolled hypertension

Variables	Controlled Hypertension (n; %)	Uncontrolled Hypertension (n; %)	95%CI	P-value
Family history				
Negative	90(63.38)	132(70.59)	1	
Positive	52(36.62)	55(29.41)	1.91(1.04-3.49)	0.034
Total Cholesterol				
<5.2 mmol/L	101(71,13)	131(70.05)	1	
≥5.2 mmol/L	41(28.87)	56(29.94)	1.12(0.53-2.37)	0.759
HDL-C				
≥1 mmol/L	100(70.42)	59(31.55)	1	
<1 mmol/L	42(29.58)	128(68.45)	0.13(0.07-0.24)	**<0.001**
LDL-C				
<2.6 mmol/L	69(48.59)	86(45.99)	1	
≥2.6 mmol/L	73(51.41)	101(54.01)	0.83(0.42-1.64)	0.596
Triglycerides				
<1.7 mmol/L	65(45.77)	74(39.57)	1	
≥1.8 mm0/L	77(54.23)	113(60.43)	0.74(0.40-1.37)	0.342
Drug combinations				
Thiazide	23(16.20)	21(11.23)	1	
Thiazide+CCB+ACEI	23(16.19)	47(25.13)	1.37(0.40-4.72)	0.610
Thiazide+CCB+ACEI+β-blocker	04(2.82)	14(7.49)	0.65(0.20-2.14)	0.485
Other	92(64.79)	105(56.15)	5.09(0.15-173.39)	0.366
Number of Drugs				
1	38(26.76)	31(16.58)	1	
2	69(48.59)	68(36.36)	1.13(0.04-32.33)	0.943
3	30(21.13)	73(39.04)	1.55(0.06-39.10)	0.788

4	05(3.52)	15(8.02)	6.33(0.22-181.92)	0.281
Diabetes				
No	80(56.34)	116(62.03)	1	
Yes	62(43.66)	71(37.97)	1.18(0.65-2.14)	0.587
Obesity				
No	66(46.48)	56(29.95)	1	
Yes	76(53.52)	131(70.05)	0.35(0.19-0.66)	**0.001**
Physical Activity				
Inactive	121(85.21)	127(67.91)	1	
Active	21(14,79)	60(32.09)	0.18(0.08-0.43)	**<0.001**

HDL-C=High density lipoprotein cholesterol; LDL-C=Low density lipoprotein cholesterol

In the multiple logistic regression (crude and adjusted) regression model analysis (Table 3), the categories were merged to create a binary outcome for each of the variables: education, employment and age. In the final model, obesity (1.29-3.20), physical activity (1.56-4.75), low HDL-C (3.21-8.30), combination regimen (thiazide, calcium channel blockers and angiotensin converting enzyme inhibitors) (1.01-3.17) and being on two (0.09-0.83) or three drugs (0.11-0.95) were the independent and significant determinants of uncontrolled hypertension. However, after adjusting for confounding factors (level of education, ethnicity, smoking status, alcohol use, fruit and vegetable consumption, family history of hypertension, total cholesterol, triglycerides and LDL-C), obesity (1.66-5.05), physical activity (2.15-10.65) and low HDL-C (3.33-9.60) were the significant and independent determinants of uncontrolled hypertension in the cohort. Individuals who were obese were three times more likely to have uncontrolled hypertension compared to those who were not obese. Likewise, participants who were physical active were close to five times more likely to have uncontrolled hypertension compared to those who did not engage in physical activity. Individuals with low HDL-C were close to six times more likely to have uncontrolled hypertension compared to those with normal HDL-C.

Table 3: Adjusted and unadjusted logistic regression models showing the factors associated with uncontrolled hypertension

Variables	Unadjusted odds ratios (95% CI)	Adjusted odds ratios (95% CI)
All		
Gender		
Male	1.03 (0.59-1.80)	1.00 (0.51-1.94)
Female	1	1
Age		
<55	0.94 (0.59-1.51)	0.85 (0.48-1.48)
≥55	1	1
Employment status		
Unemployed	1.49 (0.93-2.41)	1.47 (0.83-2.62)
Employed	1	1
Duration since diagnosis		
>5 years	0.69 (0.42-1.13)	1.99 (0.94-4.20)
5 years and below	1	1
Salt intake		
Increased	0.91 (0.47-1.74)	1.64 (0.72-3.71)
Low-Moderate	1	1
Diabetes		
Yes	0.79 (0.51-1.23)	0.86 (0.50-1.48)
No	1	1
Obesity		
Yes	2.03 (1.29-3.20)*	2.90 (1.66-5.05)***
No		
Physical Activity		
Active	2.72 (1.56-4.75)***	4.79 (2.15-10.65)***
Inactive	1	
HDL-C		
<1mmol/L	5.17 (3.21-8.30)***	5.66 (3.33-9.60)***
≥1 mmol/L	1	1
Drug Combinations		
Thiazide	0.80 (0.42-1.54)	1.32 (0.42-4.12)
Thiazide+CCB+ACEI	1.79 (1.01-3.17)*	0.67 (0.23-1.92)
Thiazide+CCB+ACEI+ β-Blocker	3.07 (0.98-9.64)	2.00 (0.07-57.94)
Other	1	1
Number of Drugs		
1	0.27 (0.09-0.83)*	0.38 (0.02-9.92)
2	0.33 (0.11-0.95)*	0.54 (0.02-12.44)
3	0.81 (0.27-2.43)	1.71 (0.07-43.64)
4	1	1

***p-values <0.001; *p-values<0.05; CI: Confidence Interval; HDL-C=High density lipoprotein cholesterol; LDL-C=Low density lipoprotein cholesterol.

Discussion

Hypertension is an independent risk factor for cardiovascular diseases and all causes of premature deaths [2,7,18]. As such, the control of hypertension is essential in lowering cardiovascular and mortality risk in patients [18,21]. However, the prevalence and factors that determine uncontrolled hypertension in rural communities of South Africa are understudied and the actual burden of the disease is poorly understood. This study therefore aimed to assess the prevalence, socio-demographic and clinical determinants of uncontrolled hypertension among patients receiving healthcare in three government facilities serving the rural areas of Mkhondo municipality in the Mpumalanga province, South Africa.

South Africa has the highest prevalence of hypertension in Southern Africa [4]. Despite the high prevalence, the level of awareness and control of the disease is low among impoverished communities [3]. In the current study, the overall prevalence of uncontrolled hypertension was 56.83%. In comparison to other studies conducted in Africa, the figure presented in this study was slightly lower than those reported in Zimbabwe (61.0%) [25] and Nigeria (60.0%) [26], but higher than the 52.7% reported in Ethiopia [27]. In comparison to other studies conducted in different parts of South Africa, the result is lower than the 75.5% that was reported in the rural Eastern Cape province [12]; however, higher than the 13.5% prevalence that was reported in the South African National Health and Nutrition Examination Survey [14]. It is important to note that the wide disparity observed between the two results could be explained by the large sample of non-hypertensive individuals in the national survey, whilst the current study was exclusive to hypertensive patients.

In the current study, the presence of obesity was significantly associated with uncontrolled hypertension. These findings are supported by studies conducted in Ethiopia [13,27], China

108

[28] and Zimbabwe [29], where excessive body weight was statistically associated with the incidence of uncontrolled hypertension [28,29]. Moreover, it has been shown that a modest weight-loss will not only decrease blood pressure, but will also have a favourable impact on obesity related risk factors [29,30]. Obesity is traditionally defined as the abnormal accumulation of body fat $\geq 20\%$ over the individual's ideal body weight and assessed as BMI $\geq 30kg/m^2$ [29]. The increasing prevalence of obesity has been identified as the most important risk factor for a number of life threatening conditions, including hypertension [31,32]. Patients with obese-related hypertension often present with increased blood volume, high levels of circulating aldosterone, insulin resistance and obstructive sleep apnea syndrome [33,34]. A number of studies have identified that there is a direct and apparent dose-response relationship between an increase in BMI and blood pressure [35].

In the current study, engagement in physical activity was associated with the higher odds of having uncontrolled hypertension. It should be noted that the activity level of the participants was obtained by self-report and there was no standardized measure of the intensity of the physical activity. In addition, there is lack of formal recreational centres (gymnasium) for graded exercises in the study setting. Also, other risk factors such as excessive salt intake may have masked the beneficial effect of exercise on blood pressure control in this population. A study conducted in Ethiopia showed that non-adherence to physical activity was associated with uncontrolled hypertension [13]. In addition, a study conducted in China showed that lack of physical activity was significantly associated with uncontrolled hypertension [28]. The exact mechanism through which physical activity alleviates high blood pressure has not yet been fully elucidated. In addition, optimal prescription of physical activity for hypertension control is not known. Current hypotheses suggest that there is a link between hypertension control and alterations in insulin sensitivity, autonomic nervous system function and vasoconstriction regulation [13]. There seems to be more evidence supporting the beneficial effects of physical

activity on hypertension prevention than those refuting it. Engagement in physical activity for at least 30 – 45 minutes, five times per week is associated with a lower incidence of hypertension [36]. In the last few decades, there has been a great deal of evidence on the protective effect of physical activity on the development of hypertension [36,37]. As a result, physical activity has become the most common lifestyle modification that is often recommended after an individual has been diagnosed with hypertension [37].

Furthermore, the current study found an association between decreased HDL-C (<1mmol/L) (dyslipidaemia) and uncontrolled hypertension. Dyslipidemia comprises having abnormality in LDL-cholesterol, HDL-cholesterol and triglyceride levels (38). Abnormalities in lipid levels is believed to be a key player in the development of coronary heart disease and may be present long before other risk factors occur [38]. Also, the co-existence of hypertension and dyslipidemia is often observed in clinical practice [39] and the risk of cardiovascular events in individuals with concomitant hypertension and dyslipidemia is increased [38,40]. A recent study, demonstrated that low HDL-C levels are associated with an increased risk of hypertension [41]. These findings were similar to observations made in a study conducted in the United States of America, where low levels of HDL-C among the elderly were associated with hypertension and an increased risk of cardiovascular disease [42]. To date, prospective studies that have demonstrated the relationship between controlled blood pressure and plasma lipid levels, particularly HDL-C, are lacking. Therefore, the finding of the association between low HDL-C and uncontrolled hypertension in the present study requires further investigations.

Anti-hypertensive drugs are prescribed mainly to reduce blood pressure and complications associated with the disease. Studies have shown that following clinical guidelines in practice improve treatment outcome [43]. Additionally, clinical guidelines including the South African hypertension guideline recommend the use of multiple drugs to effectively control blood

pressure and reduce the possibility of hypertension-related complications [24]. It is important to note that all the participants in this study had been on anti-hypertensive therapy for at least a year prior to the study. The high prevalence of uncontrolled hypertension occurred, irrespective of the number and drug combinations administered. A plausible explanation could be non-adherence to treatment among the study participants. However, the level of adherence to treatment was not explored in the current study. Irrespective of the findings in this study, it is important for clinicians to follow evidence-based guidelines in prescribing anti-hypertensive drugs in order to improve treatment outcomes in these patients.

Limitations

This study has provided very important insights on the treatment outcome (blood pressure) of individuals attending health facilities for hypertension in the region, but the limitations of the study cannot be ignored. Causal association of the determinants cannot be ascertained, given the cross-sectional design method adopted in the study. Also, the lifestyle assessment depended largely on self-report measures which are subject to social desirability bias. In addition, the urinary sodium excretion level could have added more objectivity on the consumption of salts among the study participants.

Conclusion

We found a high prevalence of uncontrolled hypertension in the study setting, possibly attributed to obesity, physical activity and dyslipidaemia. All these determinants should be addressed decisively through collaborative efforts of individuals, clinicians and health authorities in achieving the treatment blood pressure targets in the study population. To the best of our knowledge, this study is the first to report the prevalence of uncontrolled hypertension with an association between it and low HDL-C, inadequate physical activity, obesity among residents of Mkhondo municipality. Furthermore, this study has highlighted the

need to implement more aggressive health strategies targeting the identified determinants as well as blood pressure of the patients in order to improve the overall outcomes of care in the rural communities of Mkhondo municipality of Mpumalanga province.

List of Abbreviations

DM=diabetes mellitus; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; LCDs=non-communicable diseases; NHLS=National Health Laboratory Services; SEMDSA=Society of Endocrinology, Metabolism and Diabetes of South Africa; TC=total cholesterol; TG=triglyceride

Declarations

Ethical approval and consent to participate

The study protocol was approved by the Research Ethics Committee of the University of the Western Cape. The Mpumalanga Department of Health and Piet Retief hospital clinical governance gave permission for the implementation of the study protocol across the three sites. The objectives of the study were explained and written informed consent was obtained from each participant. The research process followed the Helsinki Declaration and the rights of individuals to privacy and confidentiality were respected throughout the period of the study. Participation in the study was voluntary and no compensation was offered to any of the participants.

Consent to publish

Not applicable for this paper.

Availability of data and materials

All the study materials and data are available from the corresponding author, upon reasonable request.

Competing interests

The authors declare no conflict of interest.

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Authors' Contributions

CM, BP, JJO and MB conceptualised, designed and implemented the study protocol. CM and OVA analysed the data and drafted the manuscript. All authors revised and approved the final draft of the manuscript for submission.

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Chapter 4

Genomic Association of Single Nucleotide Polymorphisms with Blood Pressure Response to Hydrochlorothiazide among South African Adults with Hypertension

4.1 Introduction

Little is known about the influence of genetic factors on blood pressure response to hypertensive treatment among Africans. The manuscript presented in this chapter describes Single Nucleotide Polymorphisms (SNPs) in hydrochlorothiazide associated genes and further assesses their correlation with blood pressure control among South African adults living with hypertension. Such information is relevant in the context of personalised hydrochlorothiazide treatment for African patients. This manuscript addresses objective 2.

4.2 Publication details

Tittle	Genomic Association of Single Nucleotide Polymorphisms with Blood Pressure Response to Hydrochlorothiazide among South African Adults with Hypertension
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4.4 Manuscript: Genomic Association of Single Nucleotide Polymorphisms with Blood Pressure Response to Hydrochlorothiazide among South African Adults with Hypertension

Abstract

This study describes single nucleotide polymorphisms (SNPs) in hydrochlorothiazide associated genes and further assesses their correlation with blood pressure control among South African adults living with hypertension. A total of 291 participants belonging to the Nguni tribes of South Africa on treatment for hypertension were recruited. Nineteen SNPs in hydrochlorothiazide pharmacogenes were selected and genotyped using MassArray. Uncontrolled hypertension was defined as blood pressure ≥140/90 mmHg. The association between genotypes, alleles and blood pressure response to treatment was determined by conducting multivariate logistic regression model analysis. Majority of the study participants were female (73.19%), Xhosa (54.98%) and had blood pressure ≥140/90 mmHg (68.73%). Seventeen SNPs were observed among the Xhosa tribe and two (rs2070744 and rs7297610) were expressed among Swati and Zulu participants. Furthermore, Allele T of rs2107614 (AOR=6.69; 95%CI 1.42-31.55) and C of rs2776546 (AOR=3.78; 95%CI 1.04-13.74) were independently associated with uncontrolled hypertension. Whilst rs2070744 TC (AOR=38.76; 95%CI 5.54-270.76), CC (AOR=10.44; 95%CI 2.16-50.29) and allele T of rs7297610 (AOR=1.86; 95%CI 1.09-3.14) were significantly associated with uncontrolled hypertension among Zulu and Swati participants. We confirmed the presence of SNPs associated with hydrochlorothiazide, some of which were significantly associated with uncontrolled

hypertension in the study sample. Findings open doors for further studies on personalized therapy for hypertension in the country.

Keywords: Uncontrolled hypertension; Hydrochlorothiazide; South Africa; Single nucleotide polymorphism

Introduction

Hypertension is the leading cause of death globally, accounting for 10.4 million deaths per year [1]. Furthermore, an estimated 1.13 billion people world-wide have been diagnosed with hypertension and most reside in low-middle income countries. In South Africa, the highest rate of hypertension has been reported among individuals aged 50 years and above, with almost eight out of ten people in this age group having being diagnosed with high blood pressure [2]. In addition, the Heart and Stroke Foundation of South Africa reported that one in three South Africans 15 years and older are hypertensive [2]. The high burden of hypertension among South Africans is accompanied by low control rates as well as adverse cardiovascular disease risk [2,3]. While epidemiological studies have improved our understanding of the environmental factors associated with hypertension control, more especially with regards to physical activity and diet, the role of genetics in this setting remains unclear. Therefore, it is critical to explore genetic factors with regards to hypertension control, in order to establish genetic-based initiatives that could be applied in medical practice to reduce the burden of hypertension and improve treatment outcome among patients.

Hydrochlorothiazide (HCTZ) is a thiazide diuretic that is indicated for the treatment of hypertension [4]. The drug has been shown to lower blood pressure by acting on the kidneys to reduce sodium (Na^+) reabsorption in the distal convoluted tubule [4,5]. Although, HCTZ has been used as a first-line drug for the treatment of hypertension for over six decades, blood pressure response to the drug is highly variable [6,7]. As such, pharmacogenomic studies have

investigated genetic polymorphisms that could account for the inter-individual variability that is observed across individual patients as well as diverse population groups. Single nucleotide polymorphisms (SNPs) in genes such as protein kinase C alpha (*PRKCA*), lysine deficient protein kinase 1(*WNK1*) beta-2 adrenergic receptor (*ADRB2*), and nitric oxide synthase 3 (NOS3) have been of particular interest due to the role they play in blood pressure control [8–10].

The *PRKCA* gene encodes an enzyme that plays an important role in the modulation of ions channels [10]. I*n vivo* studies suggest that this enzyme may be a fundamental regulator of cardiac contractility and Calcium (Ca_2^+) handling in myocytes [11]. On the other hand, the *WNK1* encodes for a ubiquitously expressed protein that regulates vasoconstriction and blood pressure response [12,13]. A study conducted among Caucasian hypertensive participants showed that an intronic SNP, rs16960228, in PRKCA is an important predictor of HCTZ blood pressure response. The study further demonstrated that rs16960228 A-allele carriers had a greater blood pressure response compared to GG carriers [14]. Also, hypertensive patients with the CC genotype of rs4791040 showed a greater reduction of diastolic blood pressure as compared to carriers of CT and TT genotypes following HCTZ treatment [14]. Inversely, hypertensive carriers of the CC genotype of rs2277869 (*WNK1)* showed increased ambulatory blood pressure as compared to carriers of CT and TT genotypes [8]. Whereas genotypes (CC and CT) of rs2107614 of the *WNK1* gene were associated with greater reduction in whole day ambulatory blood pressure among patients with essential hypertension who were treated with HCTZ [8].

The *ADRB2* and *NOS3* genes are central components of the renin–angiotensin system (RAS) that controls blood pressure by regulating the volume of fluids in the body [7,15]. As such, polymorphisms in these genes might influence blood pressure control. A study conducted in a

cohort comprised of 50% individuals of African origin, showed that the AA and AG genotypes of rs2400707 (*ADRB2*) were associated with increased reduction in whole day ambulatory blood pressure following hydrochlorothiazide treatment [8]. On the other hand, it was shown that hypertensive carriers of the CC genotype of rs2070744 (*NOS3*) who were treated with anti-hypertensive drugs including diuretics may have an increased risk for resistance to medication as compared to patients with the CT or TT genotype [16]. However, a direct association of the genotypes of rs2070744 (NOS3) with blood pressure response to HCTZ is yet to be established.

The *YEATS4* gene encodes the protein GAS41, which has been shown to mediate RNA transcription and cell viability [17]. Unlike the ADRB2, *PRKCA* and *WNK1* genes, there was no reference found in the literature that connects the *YEAST4* gene with pathways associated with hypertension or drug response. However, variation at rs7297610, where CC genotypes were associated with greater blood pressure responses to HCTZ in comparison to T-allele carriers. It was further demonstrated that such an association was absent among atenolol-treated participants [18]. Therefore, these finding suggest that there could be a potential mechanism where *YEAST4* could affect blood pressure response to thiazide diuretic medication. However, further research is needed to verify this association.

Pharmacogenomics has progressed and matured into an efficient and effective tool for mapping genes underlying human phenotypes associated with drug response. This tool holds a promise of using genome-based technologies to improve health by effectively treating diseases including hypertension [19–22]. However, pharmacogenomics is still at its infancy in the developing world, and little is known about the influence of genetic factors on blood pressure response to hypertensive treatment among Africans. In this study, we describe single nucleotide polymorphisms (SNPs) in hydrochlorothiazide associated genes and further assesses their correlation with blood pressure control among South African adults living with hypertension.

Materials and Methods

Ethical approval

The Senate Research Committee of the University of the Western Cape approved the study protocol (Ethics approval number: BM/16/5/19). Permission to implement the study was granted by the clinical governance of the respective hospitals in the Eastern Cape and Mpumalanga Provinces. Participants were issued with a research information sheet detailing the study; and it was made available in three indigenous languages (SiSwati, IsiXhosa and IsiZulu). Each participant indicated their voluntary participation by signing a consent form. The rights to privacy and confidentiality of medical information of each participant were honored during and after the study.

Patient Selection

A total of 291 Nguni (Xhosa, Swati and Zulu) patients attending chronic care for hypertension were recruited consecutively between January 2019 and June 2019, from Cecilia Makiwane Hospital (East London, Eastern Cape), Piet Retief Hospital, Thandukukhanya Community Health Center and Mkhondo Town Clinic (Mkhondo, Mpumalanga). Participants were eligible for participation if they were 18 years or older and were on continuous treatment for hypertension for at least a year prior to the study. Individuals who were bedridden, pregnant and unable to give consent were excluded from the study.

Data collection

A trained research nurse measured the blood pressure (BP) of each participant by using a validated automated digital BP monitor (Macrolife BP A 100 Plus model) according to standard protocols. Thereafter, BP was recorded in triplicate and the average was used to categorise participants into two groups: controlled (blood pressure <140/90 mmHg) and

uncontrolled (blood pressure ≥140/90 mmHg). DNA samples were collected in the form of buccal swabs and stored at -20◦ C until they were processed.

The age, ethnicity, smoking status and salt-intake were self-reported by each participant and documented in a proforma designed for this study. The number and type of anti-hypertensive drugs prescribed for each participant was retrieved from their clinical records.

DNA isolation

Genomic DNA was extracted from buccal swab samples using a standard salt-lysis procedure [32]. Briefly, DNA samples were incubated in lysis buffer at 62 °C overnight. Thereafter, DNA was precipitated with NaCl followed by the addition of 75% ice-cold ethanol and incubated at -20 °C overnight. Precipitated DNA was purified using 70% ethanol and re-suspended in nuclease-free water. Samples were stored in 2 ml Eppendorf tubes at -20 °C until further use. DNA was quantified using a NanoDrop™ 2000/2000c Spectrophotometers (Thermo Scientific™) and Gel Doc™ EZ Gel Documentation System (BIO-RAD, USA).

Genotyping

Two multiplex MassARRAY systems (Agena BioscienceTM) were designed and optimised by Inqaba Biotechnical Industries (Pretoria, South Africa) in January 2017. Each multiplex was used to genotype selected SNPs, using an assay that is based on a locus-specific PCR reaction. This reaction is followed by a single base extension using the mass-modified dideoxynucleotide terminators of an oligonucleotide primer, which anneals upstream of the site of mutation. Matrix Assisted Laser Desorption/Ionization - time-of-flight (MALDI-TOF) mass spectrometry was used to identify the SNP of interest.

Statistical analysis

Statistical analyses were performed using IBM Statistical Package for Social Science (SPSS) Version 25 for Windows (IBM Corps, Armonk, New York, USA). The general characteristics of the participants were expressed as frequency (percentages). The associations between alleles, genotypes and blood pressure response to hydrochlorothiazide were assessed by multivariate logistic regression model analysis (unadjusted and adjusted odds ratios) and their 95% confidence intervals. A p-value less than 0.05 was considered statistically significant. Minor allele frequency (MAF) and Hardy-Weinberg equilibrium (HWE) tests were calculated using Genetic Analysis in Excel (GenAlEx) Version 6.5.

Selection of pharmacogenomics biomarkers

Nineteen SNP previously associated with hypertension or hydrochlorothiazide efficacy were selected using Pharmacogenomics Knowledge Base [33], Ensembl [23] as well as an extensive survey of recent literature. Selected SNPs were in genes that are indirectly or directly involved in the pathways associated with the blood pressure lowering effect of hydrochlorothiazide on hypertension exhibiting Pharmagkb evidence rating of at least 3.

Results

General characteristics of the study

A total of 291 individuals with hypertension participated in this study, of whom 73.19% (n=213) were female and 26.04% (n=78) were male. What is the mean age (SD) of the participants? The cohort was composed of individuals belonging to the Xhosa (n=160), Zulu (n=112) and Swati (n=19) tribes of South Africa. The majority of the participants were non-smokers (67.35%), consumed low-moderate salt (81.44%) and had blood pressure ≥140/90 mmHg (68.73%) (Table 1).

Table 1: General characteristics of the study cohort

Variables	All Participants (n; %)	Males (n; %)	Females (n; %)
All	291(100)	78(26.04)	213(73.19)
Age (Years)			
18 – 25	01(0.34)	01(1.28)	0(0.00)
26 – 35	08(2.75)	02(2.56)	06(2.82)
36 – 45	19(6.52	07(8.97)	12(6.63)
46 – 55	52(17.87)	14(17.95)	38(17.84)
56 – 65	120(41.24)	25(32.05)	95(44.60)
$\geq$66	91(31.27)	29(37.18)	62(29.11)
Ethnicity			
Zulu	112(38.49)	19(24.36)	93(43.66)
Swati	19(6.53)	03(3.85)	16(7.51)
Xhosa	160(54.98)	56(71.79)	104(48.83)
Smoking status			
Never Smoked	196(67.35)	30(38.46)	166(77.93)
Ever Smoked	95(32.65)	48(61.54)	47(22.07)
Salt intake			
Low-Moderate	237(81.44)	58(74.36)	179(84.04)
Increased	54(18.56)	20(25.64)	34(15.96)
Blood Pressure			
<140/90 mmHg	91(31.26)	16(20.51)	75(35.21)
$\geq$140/90 mmHg	200(68.73)	62(79.49)	138(64.79)
Drug Regime			
HCTZ Alone	63(21.65)	20(25.64)	43(20.19)
HCTZ + 1 Drug	127(43.64)	26(33.33)	101(47.42)
HCTZ+ 2 Drugs	98(33.68)	30(38.46)	68(31.92)
HCTZ + 3 Drugs	03(1.03)	02(2.56)	01(0.47)

Expression patterns of Single nucleotide polymorphisms

Nineteen SNPs were selected and their expression patterns were assessed across three populations (Swati, Xhosa and Zulu). Seventeen out of nineteen SNPs were exclusively expressed among the Xhosa tribe (n=160), the remaining two (rs2070744 and rs7297610) were expressed among Swati and Zulu participants. Majority of the seventeen SNPs detected among the Xhosa tribe demonstrated an expression frequency above 90%, with variants rs4791040 and rs5051 showing an expression frequency of 73.10% (n=117) and 68.75% (n=110), respectively. Variant 2070744 and rs5051 showed a 100% expression efficiency among Swati (n=19) and Zulu (n=112) participants, of whom 51.17% (n=109) were female, and 44.44% (n=40) were aged 60 years and above (Table 2).
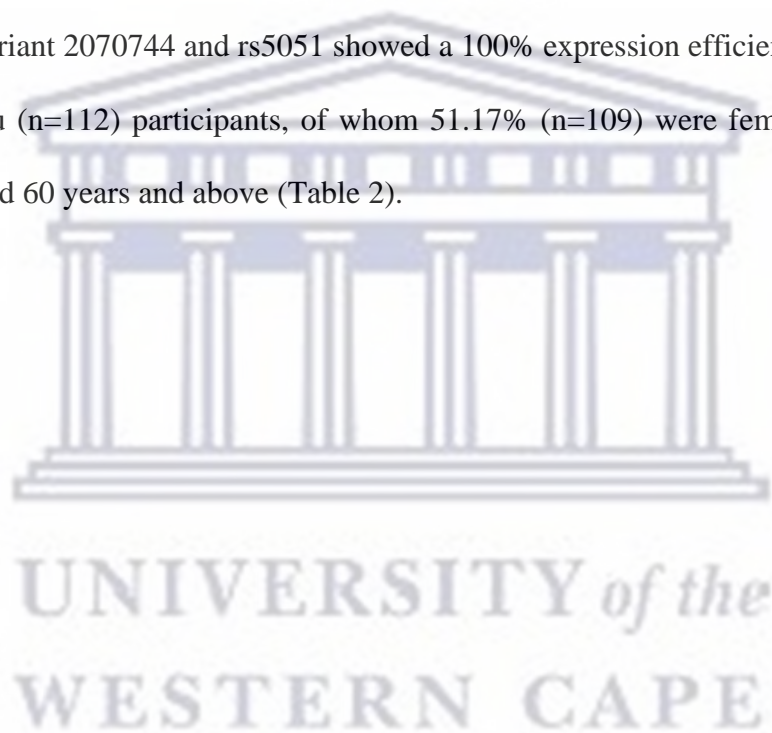
Table 2: Distribution patterns of selected Single nucleotide polymorphisms (SNPs)

dbSNP	Gene	Ethnic Groups			Gender		Age		
		Zulu (n; %)	Swati (n; %)	Xhosa (n; %)	Male (n; %)	Female (n; %)	<55 Years	55-65 Years	>65 Years
All		112(38.48)	19(6.52)	160(54.98)	78(26.80)	213(73.19)	80(27.49)	121(41.58)	90(30.93)
rs11189015	SLIT1								
Yes		-	-	155(96.88)	54(69.23)	101(47.42)	43(53.75)	62(51.24)	50(55.56)
No		112(100)	19(100)	05(3.10)	24(30.77)	112(52.58)	37(46.25)	59(48.76)	40(44.44)
rs1458038	FGF5								
Yes		-	-	156(97.50)	55(70.51)	101(47.42)	45(56.25)	62(51.24)	49(54.44)
No		112(100)	19(100)	04(2.50)	23(29.49)	112(52.58)	35(43.75)	59(48.76)	41(45.56)
rs16960228	PRKCA								
Yes		-	-	158(98.75)	56(71.79)	102(47.89)	47(58.75)	63(52.07)	48(53.33)
No		112(100)	19(100)	02(1.25)	22(28.21)	111(52.11)	33(41.25)	58(47.93)	42(46.67)
rs17010902	APOA5								
Yes		-	-	152(95.00)	54(69.23)	98(46.01)	44(55.00)	61(50.41)	47(52.22)
No		112(100)	19(100)	08(5.00)	24(30.77)	115(53.99)	36(45.00)	60(49.59)	43(47.78)
rs2106809	ACE2								
Yes		-	-	157(98.10)	54(69.23)	103(48.36)	45(56.25)	62(51.24)	50(55.56)
No		112(100)	19(100)	03(1.90)	24(30.77)	110(51.64)	35(43.75)	59(48.76)	40(44.44)
rs2107614	WNK1								
Yes		-	-	155(96.90)	55(70.51)	100(46.95)	47(58.75)	62(51.24)	46(51.11)
No		112(100)	19(100)	05(3.10)	23(29.49)	113(53.05)	33(41.25)	59(48.76)	44(48.89)
rs2269879	DOT1L								
Yes		-	-	156(97.50)	54(69.23)	102(47.89)	44(55.00)	63(52.07)	49(54.44)
No		112(100)	19(100)	04(2.50)	24(30.77)	111(52.11)	36(45.00)	58(47.93)	41(45.56)
rs2277869	WNK1								
Yes		-	-	156(97.50)	55(70.51)	101(47.42)	46(57.50)	61(50.41)	49(54.44)
No		112(100)	19(100)	04(2.50)	23(29.49)	112(52.58)	34(42.50)	60(49.59)	41(45.56)
rs2400707	ADRB2								

Yes		-	-	157(98.10)	56(71.79)	101(47.42)	45(56.25)	63(52.07)	49(54.44)
No		112(100)	19(100)	03(1.90)	22(28.21)	112(52.58)	35(43.75)	58(47.93)	41(45.56)
rs2776546	*CSMD1*								
Yes		-	-	158(98.75)	56(71.79)	102(47.89)	47(58.75)	63(52.07)	48(53.33)
No		112(100)	19(100)	02(1.25)	22(28.21)	111(52.11)	33(41.25)	58(47.93)	42(46.67)
rs292449	*NEDD4L*								
Yes		-	-	156(97.50)	55(70.51)	101(47.42)	45(56.25)	63(2.07)	48(53.33)
No		112(100)	19(100)	04(2.50)	23(29.49)	112(52.58)	35(43.75)	58(47.93)	42(46.67)
rs3184504	*SH2B3*								
Yes		-	-	159(99.40)	56(71.79)	103(48.36)	47(58.75)	63(52.07)	49(54.44)
No		112(100)	19(100)	01(0.60)	22(28.21)	110(51.64)	33(41.25)	58(47.93)	41(45.56)
rs4149601	*NEDD4L*								
Yes		-	-	159(99.40)	56(71.79)	103(48.36)	47(58.75)	63(52.07)	49(54.44)
No		112(100)	19(100)	01(0.60)	22(28.21)	110(51.64)	33(41.25)	58(47.93)	41(45.56)
rs4551053	EBF1								
Yes		-	-	160(100)	56(71.79)	104(48.83)	47(58.75)	63(52.07)	50(55.56)
No		112(100)	19(100)	-	22(28.21)	109(51.17)	33(41.25)	58(47.93)	40(44.44)
rs4791040	PRKCA								
Yes		-	-	110(68.75)	38(48.72)	72(33.80)	30(37.50)	42(34.71)	38(42.22)
No		112(100)	19(100)	50(31.25)	40(51.28)	141(66.20)	50(62.50)	79(65.29)	52(57.78)
rs5051	AGT								
Yes		-	-	117(73.10)	41(52.56)	76(35.68)	31(38.75)	48(39.67)	38(42.22)
No		112(100)	19(100)	43(26.90)	37(47.44)	137(64.32)	49(61.25)	73(60.33)	52(57.78)
rs6083538	ZNF343								
Yes		-	-	156(97.50)	56(71.79)	100(46.95)	46(57.50)	63(52.07)	47(52.22)
No		112(100)	19(100)	04(2.50)	22(28.21)	113(53.05)	34(42.50)	58(47.93)	43(47.78)
rs2070744	NOS3								
Yes		112(100)	19(100)	-	22(28.21)	109(51.17)	33(41.25)	58(47.93)	40(44.44)
No		-	-	160(100)	56(71.79)	104(48.83)	47(58.75)	63(52.07)	50(55.56)
rs7297610	YEAST4								

Yes		112(100)	19(100)	-	22(28.21)	109(51.17)	33(41.25)	58(47.93)	40(44.44)
No		-	-	160(100)	56(71.79)	104(48.83)	47(58.75)	63(52.07)	50(55.56)

The Minor allele frequency (MAF) observed in all three populations were compared to the Luhya people of Kenya, the Yoruba of Nigeria, Mexican from California (USA), British of Great Britain and Punjabi of India. Variant rs11189015 (33.5%), rs17010902 (59.5%), rs2106809 (88.5%) and rs2277869 (20.5%) expressed among the Xhosa tribe showed a higher MAF in comparison to the selected reference populations listed on Ensambl (23). However, the MAF of rs2269879 (32.2%), rs2400707 (37.26%) and rs1458038 (20.3%) were lower than those observed in selected world populations. The MAF of the remaining SNPs are shown in Table 3. Variant rs7297610 (52.4%) expressed among the Swati and Zulu tribe showed a higher MAF when compared to the selected world populations. The MAF observed in variant rs2070744 (14.7%) was lower than the MAF observed among British, Mexican and Punjabi populations (Table 3). None of the SNPs in this cohort deviated from Hardy–Weinberg equilibrium.

Table 3: Minor allele frequency distribution across different population groups

dbSNP	Nucleotide substitution	Feature	MAF(%)						
			Xhosa	Swati and Zulu	Yoruba	Luhya	Mexican	British	Punjabi
rs11189015	G>A>C	Intron	33.5	-	29.6	29.8	3.9	6.6	14.1
rs1458038	C>T	Intergenic	20.3	-	94.4	97.5	73.4	73.6	77.1
rs16960228	C>T	Intron	2.2	-	40.7	28.8	9.4	7.7	0.5
rs17010902	A>G	Intergenic	59.5	-	0.5	3.5	26.0	8.8	16.1
rs2106809	A>G	Intron	88.5	-	7.3	7.8	35.0	25.7	40.3
rs2107614	T>A>C	Intron	53.5	-	38.9	53.0	64.8	73.1	69.3
rs2269879	C>T	Intron	32.2	-	64.4	63.1	75.8	93.4	91.1
rs2277869	T>C	Non-coding exon	20.5	-	13.0	17.7	18.0	14.3	19.3
rs2400707	A>G>T	5 prime UTR	37.26	-	56.9	43.3	82.8	60.4	70.8
rs2776546	C>A>T	Regulatory region	48.7	-	41.2	40.4	26.6	17.0	13.0
rs292449	G>C	5 prime UTR	49.0	-	45.4	47.0	46.9	70.3	56.8
rs3184504	T>A>C>G	Missense	**100**	-	100	100	22.7	50.0	7.3
rs4149601	G>A	Splice region	51.5	-	37.5	50.0	11.7	31.3	18.8
rs4551053	G>A	Regulatory region	13.6	-	11.1	11.6	16.4	33.0	43.2
rs4791040	T>C	Intron	38.6	-	40.7	28.8	10.7	7.7	3.1
rs5051	C>A>G>T	Intron	95.7	-	94.4	89.4	68.8	36.8	62.0
rs6083538	C>T	Intron	15.0	-	7.9	9.1	46.9	47.8	37.5

http://etd.uwc.ac.za/

rs2070744	C>T	Intron	-	14.7	12.5	14.1	27.3	46.3	25.5
rs7297610		Intergenic	-	52.4	37.0	31.3	5.5	6.0	2.1

Association between SNPs and blood pressure response to hydrochlorothiazide

In the multivariate logistic regression (unadjusted) model analysis, the allele A of rs2400707 (COR=7.34; 95%CI 3.05-17.67) was independently associated with uncontrolled hypertension, although, carriers of the genotype of rs2400707 AA (COR=0.36; 95% CI 0.15-0.85) were less likely to have uncontrolled hypertension. No association was established in the remaining sixteen SNPs expressed among the Xhosa tribe. In the adjusted logistic regression model, the direction of association for the A allele of rs2400707 shifted, carriers of the A allele (OR=0.14; 95%CI 0.03-0.665) were less likely to have uncontrolled hypertension. Furthermore, carriers of allele T of rs2107614 (AOR=6.69; 95%CI 1.42-31.55) and C of rs2776546 (AOR=3.78; 95%CI 1.04-13.74) were almost seven-times and four-times more likely to have uncontrolled hypertension, respectively. On the other hand, carriers of rs4791040 allele C (AOR=0.99; 95%CI 0.01-0.60) were less likely to have uncontrolled hypertension (Table 4).
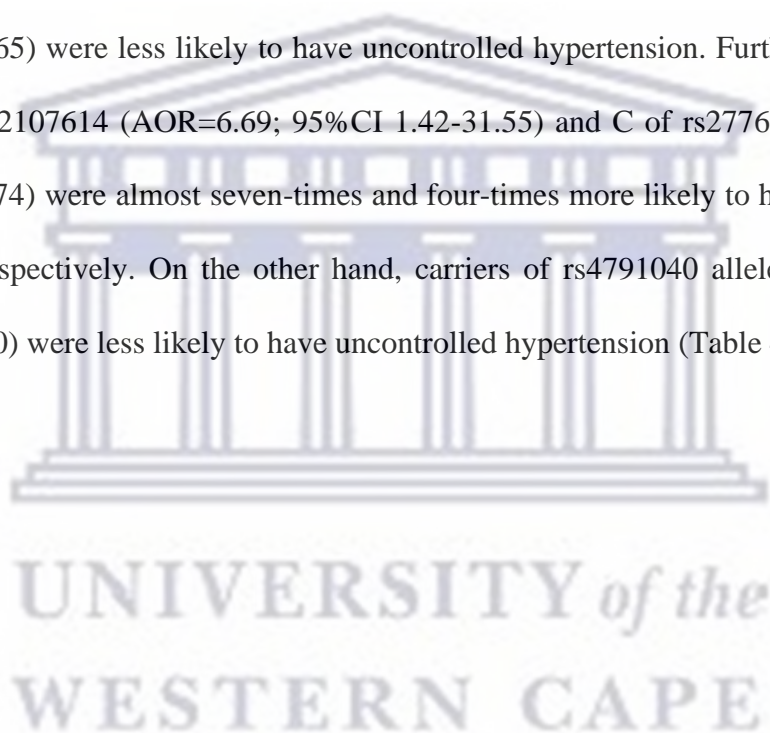
Table 4: Adjusted and unadjusted logistic regression models showing genotypes and alleles associated with blood pressure response to hydrochlorothiazide among Xhosa patients (n=160)

dbSNP	Uncontrolled HPT (n; %)	Controlled HPT (n; %)	Unadjusted odds ratios (95% CI)	p-Value	Adjusted odds ratios (95% CI)	p-Value
All	31(19.37)	129(80.63)				
rs11189015						
Genotypes						
CC	11(84.62)	02(15.38)	1		1	
GG	52(76.47)	16(23.53)	1.01(0.25-4.03)	0.982	0.82(0.30-2.22)	0.699
CG	62(83.78)	12(16.23)	1.14(0.49-2.62)		0.78(0.14-4.35)	0.786
Alleles						
G	166(80.58)	40(19.42)	1		1	
C	84(80.76)	20(19.24)	1.01(0.55-1.83)	0.969	1.65(0.40-6.70)	0.484
rs1458038						
Genotypes						
CC	89(81.65)	20(18.34)	1		1	
TT	06(66.66)	03(33.33)	0.85(0.34-2.10)	0.727	2.32(0.32-16.87)	0.403
CT	39(86.66)	06(13.33)	0.47(0.10-2.25)	0.352	1.59(0.24-10.18)	0.622
Alleles						
C	201(81.37)	46(18.62)	1		1	
T	51(79.68)	13(20.31)	1.02(0.50-2.08)	0.939	0.85(0.19-3.74)	0.832
rs16960228						
Genotypes						
CC	123(81.45)	28(18.54)	1		1	
TT	-	-	-		-	
TC	04(57.14)	03(42.85)	2.19(0.51-9.32)	0.286	0.29(0.46-1.91)	0.201
Alleles						
T	04(57.14)	03(42.85)	1		1	
C	250(80.90)	59(19.09)	1.42(0.54-3.76)	0.470	4.11(0.74-22.58)	0.104

rs17010902					
Genotypes					
GG	44(86.27)	07(13.72)	1		1	
AA	17(77.27)	05(22.73)	1.59(0.63-3.99)		0.45(0.98-2.12)	0.318
AG	61(77.21)	18(22.78)	1.33(0.44-4.01)		0.46(0.06-3.67)	0.471
Alleles					
A	95(77.23)	28(22.76)	1		1	
G	149(82.32)	32(17.68)	1.37(0.77-2.42)	0.275	0.34(0.57-2.10)	0.249
rs2106809					
Genotypes					
GG	09(75.00)	03(25.00)	1		1	
AA	109(81.95)	24(18.05)	0.75(0.13-4.25)	0.745	0.52(0.88-3.08)	0.472
AG	09(75.00)	03(25.00)	1.36(0.34-5.32)	0.657	0.40(0.75-2.16)	0.290
Alleles					
G	26(74.29)	09(25.71)	1		1	
A	227(81.65)	51(18.35)	1.48(0.65-3.34)	0.342	0.73(0.10-5.04)	0.752
rs2107614					
Genotypes					
CC	24(80.00)	06(20.00)	1		1	
TT	33(80.49)	08(19.51)	1.06(0.38-3.00)	0.901	1.17(0.37-3.68)	0.786
TC	58(78.38)	16(21.62)	1.04(0.41-2.66)	0.923	1.04(0.23-4.57)	0.952
Alleles					
C	116(80.56)	28(19.44)	1		1	
T	134(80.72)	32(19.28)	1.01(0.57-1.77)	0.970	**6.69(1.42-31.55)**	**0.016**
rs2269879					
Genotypes					
CC	60(80.33)	12(16.67)	1		1	
TT	12(75.00)	04(25.00)	1.48(0.64-3.45)	0.357	0.63(0.11-3.44)	0.599
CT	53(77.94)	15(22.06)	0.92(0.26-3.23)	0.896	1.09(0.19-6.16)	0.918
Alleles					

C	173(81.60)	39(18.40)	1		1	
T	77(77.00)	23(23.00)	0.75(0.42-1.34)	0.342	1.49(0.33-6.65)	0.597
rs2277869						
Genotypes						
CC	03(75.00)	01(25.00)	1		1	
TT	77(80.21)	19(19.79)	0.70(0.06-7.41)	0.769	0.46(0.02-7.88)	0.599
CT	45(80.36)	11(19.64)	0.97(0.42-2.22)	0.948	1.06(0.38-2.92)	0.899
Alleles						
C	51(79.69)	13(30.31)	1		1	
T	199(80.24)	49(19.76)	1.03(0.52-2.05)	0.921	0.32(0.06-1.64)	0.174
rs2400707						
GG	37(68.52)	17(31.48)	1		1	
AA	22(88.00)	03(12.00)	**0.36(0.15-0.85)**	**0.020**	0.84(0.16-4.28)	0.842
AG	67(85.90)	11(14.10)	1.24(0.31-4.83)	0.757	0.27(0.05-1.30)	0.105
Alleles						
G	141(71.57)	56(28.43)	1		1	
A	111(94.87)	06(5.13)	**7.34(3.05-17.67)**	**<0.0001**	**0.14(0.03-0.66)**	**0.013**
rs2776546						
Genotypes						
AA	32(82.05)	07(17.95)	1		1	
CC	29(82.86)	06(17.14)	1.28(0.48-3.38)	0.611	0.91(0.28-2.90)	0.878
CA	66(78.57)	18(21.43)	1.36(0.49-3.78)	0.551	1.12(0.28-4.40)	0.862
Alleles						
A	130(80.25)	32(19.75)	1		1	
C	124(80.52)	30(19.48)	1.01(0.58-1.77)	0.951	**3.78(1.04-13.74)**	**0.043**
rs292449						
Genotypes						
GG	29(74.36)	10(25.64)	1		1	
CC	35(83.33)	07(16.67)	0.68(0.27-1.73)	0.427	0.80(0.24-2.68)	0.723
GC	61(81.33)	14(18.67)	1.24(0.46-3.36	0.665	0.59(0.16-2.41)	0.422

Alleles						
G	119(73.01)	44(26.99)	1		1	
C	131(82.39)	28(17.61)	1.33(0.76-2.33)	0.308	0.34(0.09-1.21)	0.097
rs3184504						
Genotype						
CC	128(80.50)	31(19.50)	-	-	-	-
Alleles						
C	256(80.50)	62(19.50)	-	-	-	-
rs4149601						
Genotypes						
AA	34(85.00)	06(15.00)	1		1	
GG	27(77.14)	08(22.86)	0.88(0.34-2.29)	0.806	0.59(0.17-2.07)	0.417
GA	67(79.76)	17(20.24)	1.43(0.51-3.98)	0.485	0.41(0.96-1.74)	0.228
Alleles						
A	135(82.31)	29(17.68)	1		1	
G	121(78.57)	33(21.43)	1.27(0.72-2.21)	0.400	1.98(0.52-7.52)	0.312
rs4551053						
Genotypes						
GG	95(78.51)	26(21.49)	1		1	
AA	01(100)	-	-		-	
AG	33(86.84)	05(13.16)	0.55(0.19-1.57)	0.272	2.30(0.65-8.12)	0.714
Alleles						
G	223(88.49)	29(11.51)	1		1	
A	35(87.50)	05(12.50)	1.78(0.67-4.77)	0.245	0.17(0.01-2.00)	0.162
rs4791040						
Genotypes						
TT	44(86.27)	07(13.73)	1		1	
CC	19(73.08)	07(26.92)	0.87(0.32-2.15)	0.713	0.97(0.21-4.37)	0.970
TC	26(78.79)	07(21.21	1.42(0.53-3.75)	0.478	0.50(0.08-2.84)	0.437
Alleles						

T	64(75.29)	21(24.71)	1		1	
C	114(84.44)	21(15.56)	1.78(0.90-3.50)	0.095	**0.99(0.01-0.60)**	**0.012**
rs5051						
Genotypes						
CC	-	-	-		-	
TT	88(82.24)	19(17.76)	1		1	
CT	09(90.00)	01(10.00)	0.88(0.34-2.24)	0.796	1.24(0.39-3.95)	0.714
Alleles						
C	9(90.00)	01(10.00)	1		1	
T	185(82.59)	39(17.41)	0.52(0.06-4.28)	0.549	1.90(0.15-24.22)	0.618
rs6083538						
Genotypes						
CC	94(82.46)	20(17.54)	1		1	
TT	02(50.00)	02(50.00)	1.44(0.59-3.51)	0.419	0.63(0.07-5.64)	0.686
CT	29(76.32)	09(23.68)	1.08(0.91-6.19)	0.926	0.99(0.13-7.56)	0.994
Alleles						
C	217(81.58)	49(18.42)	1		1	
T	33(71.74)	13(28.26)	0.57(0.28-1.16)	0.126	2.55(0.56-11.52)	0.221

Among Zulu and Swati participants, the multivariate logistic regression model analysis showed that carriers of the genotype CC of rs2070744 (OR=4.22; 95%CI 1.15-15.47) were four-times more likely to be associated with uncontrolled hypertension. Whilst, rs2070744 TC (OR=0.10; 95%CI 0.02-0.48), rs7297610 CT (OR=0.40; 95%CI 0.16-0.98) and allele T (OR=0.60; 95%CI 0.36-0.98) carriers were less likely to have uncontrolled hypertension. After adjusting with each SNP, genotypes rs2070744 TC (AOR=38.76; 95%CI 5.54-270.76) and CC (AOR=10.44; 95%CI 2.16-50.290 were significantly associated with uncontrolled hypertension. Also, allele T of rs7297610 (AOR=1.86; 95%CI 1.09-3.14) was independently associated with uncontrolled hypertension (Table 5)

Table 5: Adjusted and unadjusted logistic regression models showing genotypes and alleles associated with blood pressure response to hydrochlorothiazide among Zulu and Swati patients (n=131)

dbSNP	Uncontrolled HPT (n; %)	Controlled HPT (n; %)	Unadjusted odds ratios (95% CI)	p-value	Adjusted odds ratios (95% CI)	p-value
All	71(54.19)	60(45.80)				
rs2070744						
Genotypes						
TT	53(55.79)	42(44.21)	1		1	
CC	02(40.00)	03(60.00)	**4.22(1.15-15.47)**	**0.030**	**10.44(2.16-50.29)**	**0.003**
TC	16(51.61)	15(48.39)	**0.10(0.02-0.48)**	**0.004**	**38.76(5.54-270.76)**	**<0.0001**
Alleles						
T	122(55.71)	97(44.29)	1		1	
C	20(45.55)	27(57.45)	0.77(0.39-1.50)	0.449	1.68(0.82-3.42)	0.151
rs7297610						
Genotypes						
CC	27(54.00)	23(46.00)	1		1	
TT	22(47.83)	24(52.17)	0.44(0.18-1.07)	0.07	2.28(8.55-6.11)	0.99
CT	21(61.76)	13(38.24)	**0.40(0.16-0.98)**	**0.045**	0.94(0.37-2.34)	0.898
Alleles						
C	75(59.52)	51(40.48)	1		1	
T	68(48.92)	71(51.08)	**0.60(0.36-0.98)**	**0.043**	**1.86(1.09-3.14)**	**0.019**

Discussion

Thiazide diuretics are among the most prescribed anti-hypertensive drugs world-wide [24]. Furthermore, this class of drugs is recommended for the initial treatment of hypertension [4]. However, pharmacogenetic markers of thiazide efficacy among African-specific populations are not well studied. As such, there is a huge knowledge gap on the effect of SNPs and blood pressure response to thiazide diuretics among populations of African origin. Therefore, this study described single nucleotide polymorphisms (SNPs) in hydrochlorothiazide associated genes and further, assessed their correlation with blood pressure control among South African adults living with hypertension.

Current research suggest that the genomes of indigenous African individuals carries the greatest depth of genetic variation compared to other population groups from around the world [25]. Thus, studying African-specific populations could help researchers understand drug response phenotypes in order to improve treatment outcomes for people living with hypertension. In the current study, nineteen SNPs previously associated with hydrochlorothiazide efficacy in individuals with hypertension were examined in 291 individuals belonging to the Zulu, Xhosa and Swati tribes (Nguni) of South Africa. Seventeen SNPs were detected among the Xhosa tribe, and only two SNPs (rs2070744 and rs7297610) were detected among the Swati and Zulu people. The minor allele frequencies of rs17010902, rs11189015, rs2277869 and rs2106809 were particularly higher among the Xhosa tribe when compared to other populations (Yoruba, Luhya, Mexican, British, Punjabi). Whilst, rs6083538 showed a lower minor allele frequency when compared to non-African populations (Mexican, British and Punjabi). The minor allele frequencies of the remainder of SNPs, were comparable to the selected African populations (Yoruba and Luhya) as well as those from other parts of the world. In addition, the minor allele frequencies of the two SNPs detected among the Swati

142

and Zulu people was also compared with other population groups. Variant rs7297610 showed a higher minor allele frequency in comparison to all the other population groups. Variant rs2070744 demonstrated a frequency similar to that of Luhya people (Kenya), however; lower than the minor allele frequencies observed across Mexican, British and Punjabi population groups. The genetic architecture of Nguni speaking tribes, has been described as fairly homogeneous, however; the finding of this study suggests that some disparities in blood pressure response to hydrochlorothiazide brought by SNPs that each tribe possess may exist. Although, this panel of SNPs does not represent the entire human genome, but at least open doors for more genetic studies in order to gain broader understanding of personalized treatment in patient care especially in individuals with hypertension. Findings from future studies with larger sample size drawn from the broader ethnically diverse population of South Africans might guide the selection and dosing of thiazide diuretics as well as other hypertensive drugs.

The *WNK1* gene encode a protein that plays an important role in renal ion transport [13]. On the other hand, the *ADRB2* gene mediates a rise in intracellular cAMP concentration, which, through smooth muscle relaxation, leads to vasodilation [4]. Blunted ADRB2 and WNK1 function have been implicated in the pathogenesis of hypertension. In this study, the T allele of rs2107614 (WNK1) was significantly associated with uncontrolled blood pressure among Xhosa participants, however; no association was established with any of the genotypes. In contrast, Turner et al (2005) showed that the genotypes CC and CT of rs2107614 (WNK1) were associated with increased reduction in whole day ambulatory blood pressure among individuals with non-complicated hypertension treated with HCTZ [8]. On the other hand, this study showed that carriers of the A allele and the AA genotype of rs2400707 (*ADRB2*) were less likely to have uncontrolled blood pressure. These observations are in line with previous findings, where the AA and AG genotypes of rs2400707 (ADRB2) were associated with increased reduction in whole day ambulatory blood pressure in individuals with essential

143

hypertension undergoing HCTZ treatment [8]. These finding indicate that polymorphisms in genes regulating renal sodium transport and smooth muscle relaxation, may predict inter-individual variability in blood pressure response to HCTZ. Furthermore, these genes as well as their SNPs may serve as therapeutic markers for individualizing thiazide treatment for hypertensive patients of African ancestry.

The variant rs2776545 occurs in the regulatory region of the CUB and Sushi multiple domains 1 (*CSMD1*) that encodes a product that functions as a complement control protein [26]. In this study, allele C of rs2776546 was associated with uncontrolled blood pressure among patients belonging to the Xhosa tribe. However, no association was established between HCTZ treatment response and the genotypes of the SNP. Conversely, a previous study showed that the A allele of rs2776546 was associated with increased response to thiazide diuretics in people with hypertension as compared to allele C. It was further demonstrated that carriers of the AA genotype of European ancestry treated with HCTZ showed a greater reduction of diastolic blood pressure as compared to patients with the AC or CC genotypes [14]. Moreover, the CSMD1 gene was associated with an increased risk of hypertension among Korean patients [27,28]. Although the role, of CSMD1 in the pathophysiology of hypertension is not completely understood, the findings of this study have brought attention to a clinically relevant loci on blood pressure response to thiazide diuretics among individuals of African ancestry, and further highlighted the need for more studies with larger sample sizes that could validate the direction of association of each allele and genotype.

The PRKCA gene is an important regulator of many physiological functions including secretion and exocytosis, modulation of ion channel (Ca^{2+} ions) gene expression and cell growth and proliferation [29] that harbors the SNP rs4791040. The current study showed that, Xhosa carriers of the C allele of rs4791040 were less likely to have uncontrolled blood

pressure. Furthermore, a previous study conducted among hypertensive patients of European origin showed that the allele T of rs4791040 was associated with decreased response to diuretics including hydrochlorothiazide as compared to allele C. It was further demonstrated that carrier of TT genotype treated with HCTZ may have decreased reduction of diastolic blood pressure as compared to patients with the CC or CT genotypes [8]. Although, no association was established between the genotypes of rs4791040 and blood pressure response to hydrochlorothiazide in the present study, the current findings have provided substantial evidence that PRKCA polymorphisms may influence blood pressure response to hydrochlorothiazide owing to their role in the modulation of ion channels.

Single nucleotide polymorphism rs2070744 is an intronic variant that sits on the NOS3 gene. In addition, rs2070744 has been implicated in the variable response of thiazide diuretics. In the present study, carriers of CC and TC genotypes (rs2070744) were more likely to have uncontrolled hypertension. However, no clear association was established between the genotypes of rs2070744 and blood pressure response to HCTZ. The wide confidence interval reflects the small sample size of participants included in this study. As such, a follow up study involving larger number of participants will provide clarity on the effect size of the associations observed in this study. Comparable trends were observed in a similar study, where hypertensive carriers of the CC genotype treated with anti-hypertensive drugs including HCTZ demonstrated an increased risk of resistant hypertension as compared to TC and TT carriers [30]. It was further demonstrated that carriers of the TC genotype may have a decreased, but not absent, risk for resistant hypertension. Moreover, the authors described resistant hypertension as uncontrolled blood pressure when treated with lifestyle measures and at least three anti-hypertensive drugs including an adequately dosed diuretic. In the present study, uncontrolled hypertension was defined as blood pressure ≥140/90 mmHg whilst on treatment. Lifestyle measurements, the number of drugs administered as well as the effect of other drugs on blood

145

pressure control were not considered. The degree of association between HCTZ treatment response and SNPs was solely measured without taking into consideration other drugs administered.

This study also investigated the effect of YEAST4 polymorphism (rs7297610) on blood pressure response to hydrochlorothiazide. The T allele of rs7297610 was independently associated with uncontrolled hypertension among Swati and Zulu patients. This study further demonstrates that that carriers of CT genotype were less likely to have uncontrolled blood pressure. The observations made in this study are in line with previous findings, where allele C was associated with increased reduction in blood pressure among individuals of mixed ancestry (African American and Afro-Caribbean) treated with hydrochlorothiazide as compared to allele T [18]. Although, other genetic and clinical factors may also influence a patient's response to hydrochlorothiazide, the study further demonstrated that patients with the TT genotype treated with hydrochlorothiazide may have a decreased response as compared to patients with the CC genotype [18]. Additionally, a haplotype made from three SNPs, rs317689/rs315135/rs7297610 (ATC) was strongly associated with greater HCTZ response, and the ACT and ATT haplotypes correlating with a smaller blood pressure response [31]. Of note, the role of YEAST4 in the development of hypertension remains elusive, however, previous findings and observations made in this study suggest that polymorphism in this gene may predict blood pressure response to thiazide diuretics among patients of African ancestry. It is also possible that the effect rs7297610 on HCTZ blood pressure response is a result of an interaction with other functional SNPs not yet known. As such, more studies need to be conducted in order to explore the functional role of YEAST4 and the mechanism in which it affects blood pressure in response to thiazide diuretics.

Conclusions

Using a candidate gene approach, we identified seventeen SNPs among the Xhosa tribe, and two SNPs among the Zulu and Swati tribes previously associated with hydrochlorothiazide efficacy and hypertension. The minor alleles of rs2107614 and rs2776546 were independently associated with uncontrolled hypertension among Xhosa participants. Furthermore, the T allele of rs7297610 was independently and significantly associated with uncontrolled hypertension among Swati and Zulu participants. This study also provided preliminary information for the association of YEAST4 polymorphisms in blood pressure response to hydrochlorothiazide. However, replication of these findings in a larger South African cohort is needed to confirm the associations observed in this study. Further elucidation of the exact mechanism in which these SNPs affect blood pressure in response to hydrochlorothiazide can ultimately aid in improving individualized anti-hypertensive therapy and the identification of new drug targets.

Availability of data

The data presented in this study is available on: https://drive.google.com/file/d/1lR-hVKeC86Ht5XDPg35wmXm-JLZ4bmNJ/view?usp=sharing

Acknowledgements

Authors **'Contributions**

CM, BP, JJO and MB conceptualised, designed and implemented the study protocol. CM and OVA analysed the data and drafted the manuscript. All authors revised and approved the final draft of the manuscript for submission.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 5

Single Nucleotide Polymorphisms in Amlodipine associated genes and their associations with blood pressure control among South African adults with Hypertension

5.1 Introduction

The lack of African-specific genomic data has slowed down the understanding of the underlying mechanisms implicated in blood pressure response to amlodipine and the generation of new leads relevant in personalised anti-hypertensive treatment for local populations of African origin. This chapter described SNPs in amlodipine associated genes and further assessed their correlation with blood pressure control among South African adults with hypertension. Objective 3 is addressed by this manuscript. Publication details

5.2 Publication details

Title	Single Nucleotide Polymorphisms in Amlodipine associated genes and their associations with blood pressure control among South African adults with Hypertension
Authors	CM Masilela, L Xhakaza, B Pearce, JJ Ongole, OV Adeniyi and M Benjeddou
Authors' Contribution	CM, BP, JJO and MB conceptualised, designed and implemented the study protocol. CM and OVA analysed the data and drafted the manuscript. All authors revised and approved the final draft of the manuscript for submission
Ethics	BM/16/5/19
Journal	Pharmacogenetics and Genomic
Status	Submitted for review (Pending initial response)

5.3 Journal information

Pharmacogenetics and Genomics is devoted to the rapid publication of research papers, brief review articles and short communications on genetic determinants in response to drugs and

other chemicals in humans and animals. The Journal brings together papers from the entire spectrum of biomedical research and science, including biochemistry, bioinformatics, clinical pharmacology, clinical pharmacy, epidemiology, genetics, genomics, molecular biology, pharmacology, pharmaceutical sciences, and toxicology.

5.4 Manuscript: Single Nucleotide Polymorphisms in Amlodipine associated genes and their associations with blood pressure control among South African adults with Hypertension.

Abstract

Objective: This study describes the single nucleotide polymorphisms (SNPs) in amlodipine associated genes and assesses their correlation with blood pressure control among South African adults with hypertension.

Methods: A total of 304 hypertensive patients on amlodipine treatment belonging to the indigenous Swati, Xhosa and Zulu population groups of South Africa were recruited between June 2017 – June 2019. Participants were categorized into: controlled (blood pressure <140/90 mmHg) and uncontrolled (blood pressure ≥ 140/90 mmHg) hypertension. Twelve SNPs in amlodipine pharmacogenes with a high PharmGKB evidence base were selected and genotyped using MassArray (Agena Bioscience™). Logistic regression was fitted to identify the significant associations between the SNPs and blood pressure control with amlodipine.

Results: The majority of the participants were females (76.64%), older than 45 years (89.13%) and had uncontrolled hypertension (52.31%). Of the 12 SNPs genotyped, five SNPs; rs1042713 (MAF=45.9%), rs10494366 (MAF=35.3%), rs2239050 (MAF=28.7%), rs2246709

(MAF=51.6%) and rs4291 (MAF=34.4%) were detected among the Xhosa participants, while none was detected among the Swati and Zulu tribal groups. Variants, rs1042713 and rs10494366, demonstrated an expression frequency of 97.55% and 79.51%, respectively. Variant TA genotype of rs4291 was significantly associated with uncontrolled hypertension. No association was established between blood pressure response to amlodipine and the remaining four SNPs.

Conclusion: This study reports the discovery of five SNPs in amlodipine genes (rs2239050, rs2246709, rs4291, rs1042713 and rs10494366) among the indigenous Xhosa speaking tribe of South Africa. In addition, the TA genotype of rs4291 was associated with blood pressure control in this cohort. These findings might open doors for more pharmacogenomic studies which could inform innovations to personalised anti-hypertensive treatment in the ethnically diverse population of South Africa.

Keywords: Amlodipine; Single nucleotide polymorphisms; South Africa; Uncontrolled hypertension

Introduction

Hypertension is one of the most potent cardiovascular risk factors that affects over a billion people world-wide [1]. The 2016 Demographic and Health Survey reported a prevalence of up to 23% (women) and 13% (men), among South Africans adults [2]. However, newer studies have reported a national prevalence of up to 60% [1]. Additionally, studies conducted in KwaZulu Natal, Eastern Cape, Gauteng, and the Limpopo province of South Africa have reported a range of prevalence between 12.4% and 52.0% [1,3–5]. Despite the high prevalence, the rate of uncontrolled hypertension is high among South African adults [6]. A recent study reported an overall prevalence of uncontrolled hypertension of 56.83% among rural dwellers of the Mpumalanga province [7]. While a 75.5% and 51.0% prevalence were reported in rural

153

Eastern Cape and KwaZulu Natal, respectively [6,8]. As hypertension care is slowly evolving to include behavioural and socio-demographic factors, a large body of evidence suggest that utilizing genetic factors to characterise response to pharmacological treatments offers a new approach to tailoring anti-hypertensive treatment to patients [9,10]. Consequently, there is a need for robust anti-hypertensive management strategies that will utilise genetic factors during drug selection and dosing in order to ensure optimal blood pressure control.

Amlodipine is a long-acting third generation calcium channel blocker (CCB) that has been shown to effectively lower blood pressure and reduce cardiovascular disease risk among hypertensive patients [11,12]. However, blood pressure response to amlodipine is highly variable and a number of studies have investigated the potential genetic polymorphisms that could account for the inter-individual variability observed across individual patients and populations [13]. These studies examined single nucleotide polymorphisms (SNPs) that occur in genes that are directly involved in the pharmacodynamics and pharmacokinetics of amlodipine such as the voltage-gated calcium channel α1C (*CACNA1C*) [14,15]. The *CACNA1C* gene encodes for an alpha-1 subunit of a voltage-dependent calcium channel that mediates the influx of calcium ions into the cell upon membrane depolarization [16]. This gene harbors SNPs (rs2239050, rs2238032 and rs527974) that have been implicated in hypertension. Both rs2238032 and rs2239050 were associated with uncontrolled hypertension among Caucasian patients [13,15]. On the contrary, Japanese carriers of the promoter variant rs527974 with uncontrolled hypertension showed increased amlodipine sensitivity [13]. Also, amlodipine is largely metabolised in the liver by the enzyme cytochrome P450 3A5, that is encoded by the gene Cytochrome P450 Family 3 Subfamily A Member 5 (*CYP3A5*) [17]. Literature suggest that Chinese carriers of CYP3A5*3/*3, CYP3A5*3 and CYP3A5*6 polymorphisms demonstrate increased amlodipine metabolism, as well as increased CYP3A enzyme efficacy [18].

In addition, SNPs that are harbored by genes that are indirectly implicated in the pharmacokinetics of the anti-hypertensive effect of amlodipine such as angiotensin converting enzyme (*ACE*) and angiotensinogen (*AGT*) have been examined [9,13]. Both *ACE* and *AGT* are central components of the renin–angiotensin system (RAS) that controls blood pressure by regulating the volume of fluids in the body [19] . Variant rs4291 of the *ACE* gene was strongly associated with the incidence of high blood pressure [9], however; its direct association with blood pressure response to amlodipine remains elusive. Similarly, African American carriers of the minor allele of rs11122576 of *AGT* gene who were undergoing amlodipine therapy showed a decreased risk of coronary heart diseases, however, no clear association with blood pressure in response to amlodipine has been established [20]. Additionally, Beta-adrenergic receptor (*ADRB2*) and nitric oxide synthase-1-adaptor protein (*NOS1AP*) are part of the sympathetic and para-sympathetic nervous systems and are known to be involved in the pathophysiology of hypertension [21,22]. The minor allele G of rs10494366 of the *NOS1AP* gene was associated with an increase in cardiovascular mortality among Caucasian users of amlodipine [22]. Furthermore, patients with the AA genotype of rs1042713 (*ADRB2*) demonstrate poor efficacy of cardiovascular drugs including ACE-inhibitors [23]. Nevertheless, no direct association with blood pressure response to amlodipine has been established for both SNPs. More studies need to be conducted in order to establish a clear association between these SNPs and blood pressure response to amlodipine. `

The discovery of SNPs has facilitated patient stratification for many diseases including hypertension, and allowed physicians to adopt pharmacogenomics-based approaches in the selection of anti-hypertensive drugs in order to improve drug efficacy among patients (24). Although, these findings have added relevant biological insights with regard to disease phenotypes, only a small fraction of published data has focused on indigenous South African populations and the genetic contribution of SNPs on therapeutic drug response, this is

particularly evident in the case of amlodipine. Moreover, the lack of African-specific genomic data has slowed down our understanding of the underlying mechanisms implicated in blood pressure response to amlodipine and the generation of new leads relevant in personalised anti-hypertensive treatment for local populations. More studies based on ethnically diverse populations are needed in order to improve and guide treatment strategies for African specific populations. The current study describes the single nucleotide polymorphisms (SNPs) in amlodipine associated genes and further assesses their correlation with blood pressure control among South African adults with hypertension.

Methods

Ethical approval

The study protocol was approved by the Senate Research Committee of the University of the Western Cape (Ethics approval number: BM/16/5/19). Permission to implement the study protocol was granted by the clinical governance of the respective hospitals in the Eastern Cape and Mpumalanga Provinces. Participants were issued with a research information sheet detailing the purpose and process of the study; it was made available in various indigenous languages (Swati, Xhosa and Zulu). Each participant signed an informed consent form as evidence of voluntary participation in the study. Participants' rights to privacy and confidentiality of medical information were respected during and after the study.

Patient Selection and data collection

A total of 304 patients with hypertension belonging to the indigenous Nguni (Swati, Xhosa and Zulu) population groups of South Africa were recruited from Cecilia Makiwane Hospital (East London, Eastern Cape), Piet Retief Hospital, Thandukukhanya Community Health Center and Mkhondo Town Clinic (Mkhondo, Mpumalanga) between June 2017 - June 2019. Participants

were eligible if they were 18 years or older and were on continuous anti-hypertensive therapy treatment for at least a year. Individuals who were bedridden, pregnant and clinically unstable were excluded from the study.

A trained research nurse measured the blood pressure (BP) of each participant using a validated automated digital blood pressure monitor (Macrolife BP A 100 Plus model) according to standard protocols. The BP was recorded in triplicate and the average was used for analysis. Patients were categorized as: controlled (blood pressure <140/90 mmHg) and uncontrolled (blood pressure ≥140/90 mmHg). DNA samples were collected in the form of buccal swabs and stored at -20∘ C.

In addition, the nurse also measured the weight of each participant to the nearest 0.1 kg using a digital scale (Tanita-HD 309, Creative Health Products, MI, USA) and height to the nearest of 0.1 cm using a mounted stadiometer. Participants wore minimal clothing and no shoes. Body Mass Index (BMI) was estimated as weight (kg) divided by height in meters squared (m^2) and was categorized based on WHO criteria as underweight, normal, overweight and obese (30 or greater kg/ m^2). The age, ethnicity, smoking status and salt-intake were self-reported by each participant and documented in a proforma designed for this study. Prescribed drugs for each participant were retrieved from their medical records. Physical activity was categorised as active if participants engaged in exercise leading to an increase in heart and respiratory rate, such as gardening, or inactive if they did not engage in any physical activity. Smoking status was categorised as never smoked or ever smoked (current smokers or have a history of tobacco use). Salt intake was determined by the survey question "do you add salt on the table while eating or on purchased take-away food? Participants who answered "yes" were placed in the increased salt intake category; participants who answered "no" were placed in the low-

moderate salt intake category. Prescribed anti-hypertensive drugs included amlodipine alone or in combination with hydrochlorothiazide, enalapril and atenolol.

Laboratory assessments

Total cholesterol, triglycerides, low-density lipoprotein and high-density lipoprotein cholesterol were assayed from venous blood samples after a minimum of eight-hour fasting by the participants. Lipid profile analysis was conducted by the National Health Laboratory Services (NHLS) of Cecilia Makiwane, Piet Retief and Ermelo Provincial hospitals in accordance with standard protocols and categorised according to the guidelines of The Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA) [25].

DNA isolation

Genomic DNA was extracted from buccal swab samples using a standard salt-lysis procedure (26). Briefly, DNA samples were incubated in lysis buffer at 62 °C overnight Thereafter, DNA was precipitated with NaCl followed by the addition of absolute ethanol and incubated at -80 °C for 30 minutes. Precipitated DNA was purified using 70% ethanol and re-suspended in nuclease-free water. Samples were stored at -20 °C until further use. DNA quantification was conducted using a NanoDrop™ 2000/2000c Spectrophotometers (Thermo Scientific™) and Gel Doc™ EZ Gel Documentation System (BIO-RAD, USA).

Selection of pharmacogenomics biomarkers

Twelve single nucleotide polymorphisms associated with blood pressure response to amlodipine were selected using the Pharmacogenomics knowledge base [27], Ensembl [28] as well as an extensive survey of recent literature. We focused on genes in pathways directly or indirectly involved in the blood pressure lowering mechanism of amlodipine exhibiting Pharmagkb evidence rating of at least 3 (Table 1).

Table 1. Selected amlodipine variants used in the design of multiplex MassARRAY panels (n=13)

SNP	GENE	Level of Evidence	Reference
rs1045642	ABCB1	3	www.pharmgkb.org
rs10494366	NOS1AP	3	www.pharmgkb.org
rs11122576	AGT	3	www.pharmgkb.org
rs12143842	AGT	3	www.pharmgkb.org
rs1799752	ACE	3	www.pharmgkb.org
rs2246709	CYP3A4	3	www.pharmgkb.org
rs2740574	CYP3A4	3	www.pharmgkb.org
rs4291	ACE	3	www.pharmgkb.org
rs2032582	ABCB1	3	www.pharmgkb.org
rs1042713	ADBR2	3	www.pharmgkb.org
rs10494366	NOS1AP	3	www.pharmgkb.org
rs2239050	CACNA 1C	3	www.pharmgkb.org
rs2238032	CACNA1C	3	www.pharmgkb.org

Genotyping

Two multiplex MassARRAY systems (Agena BioscienceTM) were designed and optimised by Inqaba Biotechnical Industries (Pretoria, South Africa) and used for the genotyping of the selected SNPs. The genotyping assay is based on a locus-specific PCR reaction that is followed by a single base extension using the mass-modified dideoxynucleotide terminators of an oligonucleotide primer, which anneals immediately upstream of the site of mutation. The SNP of interest is identified using MALDI-TOF mass spectrometry (Fig. 1).

Statistical analysis

Statistical analyses were performed using Medcalc version 2.2.0.0. The general characteristics of the participants were summarised by using simple descriptive statistics. Associations

between alleles, genotypes and blood pressure response to amlodipine were measured using unadjusted and adjusted odds ratios (ORs), 95% confidence interval (95%CI) and p-value derived from unconditional logistic regression. In the final model of the adjusted logistic regression analysis, we included rs2239050, rs2246709, rs4291, rs1042713 and rs10494366. Results for the unadjusted logistic regression model analysis were expressed as crude odds ratios (CORs) and adjusted odds ratios (AORs) for the adjusted logistic regression model analysis. A p-value less than 0.05 was considered statistically significant. Bonferroni corrected p-values were set at < 0.01. Minor allele frequency (MAF) and Hardy-Weinberg equilibrium (HWE) tests were calculated for all the SNPs using Genetic Analysis in Excel (GenAIEx) Version 6.5.

Results

Baseline characteristics of the participants

A total of 304 patients participated in the study, of which 23.36% (n=71) were males and 76.64% (n=233) were females. The majority of the study participants were older than 45 years (n=271), 85.19% (n=259) had never smoked, 77.96% (n=237) used low-moderate salt intake and 53.31% (n=159) had blood pressure ≥140/90 mmHg. Overall, 4.28% (n=13) of the participants were on amlodipine monotherapy (Table 1)

160

Table 2: General characteristics of study participants disaggregated by sex (n=304)

Variables	All Participants (n; %)	Males (n; %)	Females (n; %)
All	304(100%)	71(23.36%)	233 (76.64)
Age (Years)			
18 – 25	01(0.33)	-	01(0.43)
26 – 35	09(2.96)	05(7.04)	04(1.71)
36 – 45	23(7.56)	03(4.22)	20(8.58)
46 – 55	65(21.38)	16(22.53)	49(8.58)
56 – 65	97(31.90)	24(33.80)	73(31.33)
≥66	109(35.85)	23(32.39)	86(36.90)
Ethnicity			
Zulu	139(45.72)	25(35.21)	114(48.93)
Swati	43(14.14)	06(8.45)	37(15.87)
Xhosa	122(40.13)	40(56.34)	82(35.19)
Smoking status			
Never Smoked	259(85.19)	42(59.15)	217(93.13)
Ever Smoked	45(14.81)	29(40.85)	16(6.87)
Salt intake			
Low-Moderate	237(77.96)	52(73.24)	185(79.39)
Increased	67(22.04)	19(26.76)	48(20.60)
Blood Pressure			
<140/90 mmHg	145(47.69)	25(35.21)	120(51.50)
≥140/90 mmHg	159(52.31)	46(64.79)	113(48.50)
Drug Regime			
Amlodipine Alone	13(4.28)	04(5.63)	09(3.86)
Amlodipine + 1 Drug	113(37.17)	25(35.21)	88(37.77)
Amlodipine+ 2 Drugs	152(50.00)	36(50.70)	116(49.78)
Amlodipine + 3 Drugs	26(8.55)	06(8.45)	20(8.58)

Drugs used in combination with Amlodipine: Hydrochlorothiazide, Enalapril, Atenolol

Descriptive patterns of single nucleotide polymorphisms associated with amlodipine

Twelve SNPs were selected and their expression patterns were assessed across three indigenous South African populations (Swati, Xhosa and Zulu). Out of twelve SNPs, only five were detected among the Xhosa tribe. However, the variant alleles of the five SNPs were not detected among Swati and Zulu participants. rs2239050, rs2246709 and rs4291 were observed among all Xhosa participants (n=122). rs1042713 was observed among 79.51% (n=97) of Xhosa participants of whom 76.82% (n=63) were females and 78.18% (n=43) aged between 55-65 years. Also, rs10494366 was detected among 97.55% (n=119)) of the Xhosa participants, 97.56% (n=80) were females and 98.18% (n=54) aged between 55-65 years (Table 3).

162
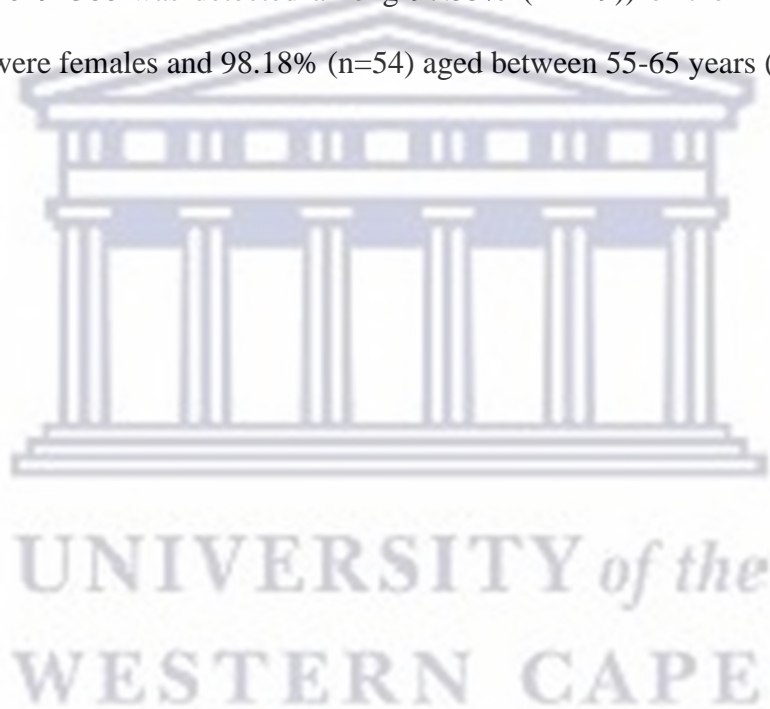
Table 3: Distribution patterns of selected Single nucleotide polymorphisms (SNPs)

dbSNP	Gene	Ethnic Groups			Gender		Age		
		Zulu (n; %)	Swati (n; %)	Xhosa (n; %)	Male (n; %)	Female (n; %)	<55 Years	55-65 Years	>65 Years
All		139(45.72%)	43(14.14%)	122(40.13%)	40(32.79%)	82(67.21%)	24(19.67%)	55(45.08%)	43(35.25%)
rs1042713	ADBR2								
Yes		-	-	97(79.51)	34(85.00)	63(76.82)	19(79.17)	43(78.18)	35(81.39)
No		139(100)	43(100)	25(20.49)	06(15.00)	19(23.17)	05(6.09)	12(21.82)	08(18.61)
rs10494366	NOS1AP								
Yes		-	-	119(97.55)	39(97.50)	80(97.56)	23(95.83)	54(98.18)	42(97.67)
No		139(100)	43(100)	03(2.46)	01(2.50)	02(2.44)	01(4.17)	01(1.82)	01(2.33)
rs2239050	CACNA1C								
Yes		-	-	122(100)	40(100)	82(100)	24(100)	55(100)	43(100)
No		139(100)	43(100)	-	-	-	-	-	-
rs2246709	CYP3A4								
Yes		-	-	122(100)	40(100)	82(100)	24(100)	55(100)	43(100)
No		139(100)	43(100)	-	-	-	-	-	-
rs4291	ACE								
Yes		-	-	122(100)	40(100)	82(100)	24(100)	55(100)	43(100)
No		139(100)	43(100)	-	-	-	-	-	-

The allelic distribution of the five SNPs did not deviate from Hardy–Weinberg equilibrium in the study cohort. The MAF observed for the selected SNPs in the Xhosa population were compared to world populations listed on Ensambl, that is, in the Luhya people of Kenya, the Yoruba of Nigeria, and African American, Mexican, British and South Asian populations. The SNPs rs1042713 (45.9%), rs10494366 (35.3%) and rs2239050 (28.7%) showed lower MAF in the Xhosa population in comparison to all selected populations. Variant rs2246709 showed a slightly higher MAF frequency in the Xhosa population (51.6%) when compared to selected world populations. In comparison to the Yoruba (22.7%) and Luhya people (23.2%), the Xhosa population (34.4%) showed a higher MAF; however, lower than British (41.4%), Mexican (44.0%) and South Asian (38.4%) populations (Table 4).
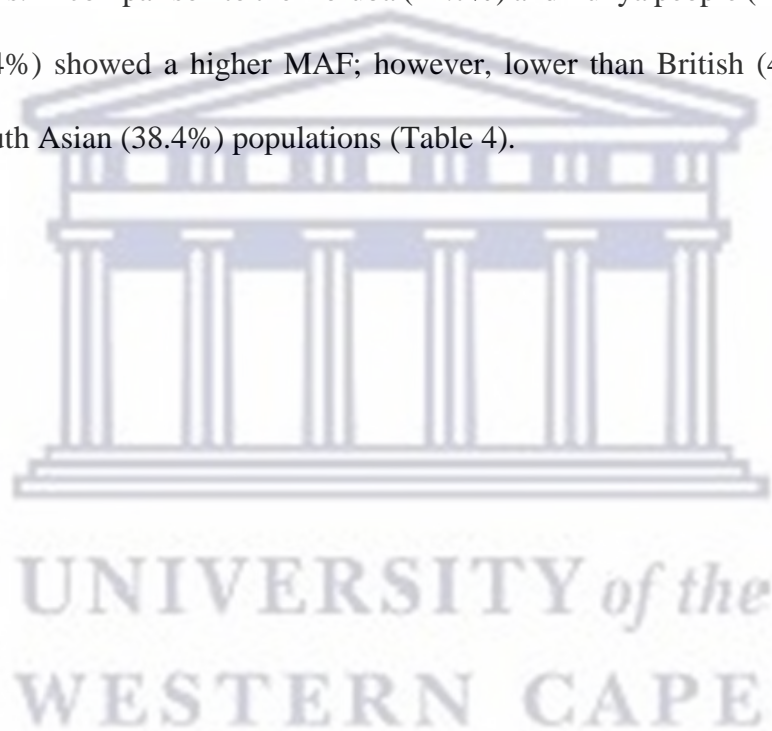
Table 4: Minor allele frequency distribution across different population groups

dbSNP	Nucleotide substitution	Feature	MAF (%)						
			Xhosa	Yoruba	Luhya	African American	Mexican	British	South Asian
rs1042713	G>A	Missense	45.9	88.0	78.8	87.7	85.9	60.4	80.7
rs10494366	G>T	Intron	35.3	88.0	86.4	77.9	57.8	50.0	60.3
rs2239050	C>G	Intron	28.7	87.5	85.9	83.6	72.7	53.8	74.5
rs2246709	A>G	Intron	51.6	13.4	12.6	14.8	14.9	20.3	14.0
rs4291	T>A	Regulatory	34.4	22.7	23.2	39.3	41.4	44.0	38.4

MAF=Minor allele frequency

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Association between SNPs and blood pressure control with amlodipine

In the multivariate (crude) logistic regression model analysis, the genotype TA (rs4291) was independently and significantly associated with uncontrolled hypertension with amlodipine treatment. After adjusting for other factors in the logistic regression model analysis, the magnitude and direction of the association remained unchanged. Individuals with genotype TA (rs4291) had lower odds of achieving blood pressure control in comparison with genotype AA (rs4291). However, the genotype GG (rs2239050) initially demonstrated an independent and significant association with controlled hypertension in response to amlodipine treatment in the crude logistic regression model analysis. After adjusting with each SNP, the effect was lost. After Bonferroni correction, TA (rs4291 remained significant with a p-value =0.004 (Table 5).

Table 5: Adjusted and unadjusted logistic regression models showing genotypes and alleles associated with blood pressure control

dbSNP	Controlled HPT (n; %)	Uncontrolled HPT (n; %)	Unadjusted odds ratios (95% CI)	p-Value	Adjusted odds ratios (95% CI)	p-Value	Bonferroni adjusted p-Value
All	20(16.39%)	102(83.60%)					
rs1042713							
Genotypes							
GG	04(15.38)	22(84.62)	1		1		
GA	10(15.15)	56(84.85)	1.29(0.32-5.21)	0.718	0.68(0.19-2.43)	0.559	0.111
AA	06(20.00)	24(80.00)	1.05(0.15-7.26	0.809	0.76(0.13-4.38)	0.768	0.153
Alleles							
G	18(15.25)	100(84.75)	1		1		
A	22(17.46)	104(82.54)	1.04(0.47-2.26)	0.928	1.12(0.47-2.63)	0.787	0.157
rs10494366							
Genotypes							
TT	13(18.06)	59(81.94)	1		1		
GT	03(13.04)	20(86.96)	0.77(0.22-2.62)	0.958	0.59(0.12-2.91)	0.520	0.104
GG	04(14.81)	23(85.19)	0.74(0.190-2.90)	0.669	0.56(0.13-2.29)	0.426	0.085
Alleles							
T	29(17.37)	138(82.63)	1		1		
G	11(14.29)	66(85.71)	0.53(0.25-1.16)	0.113	0.58(0.25-1.34)	0.208	0.041
rs2239050							
Genotypes							
CC	11(18.97)	47(81.03)	1		1		
CG	05(8.77)	52(91.23)	0.41(0.132-1.54)	0.122	0.36(0.10-1.26)	0.111	0.022
GG	04(57.14)	03(42.86)	**5.69(1.11-29.21)****	**0.003**	2.49(0.51-12.13)	0.257	0.051
Alleles							
C	27(16.61)	146(83.39)	1		1		
G	13(18.31)	58(81.69)	1.07(0.51-2.26)	0.841	0.76(0.29-1.99)	0.583	0.116
rs2246709							
Genotypes							

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GG	03(27.27)	08(72.73)	1		1		
AG	08(16.67)	40(83.33)	0.37(0.07-1.86)	0.183	0.44(0.05-3.28)	0.423	0.084
AA	09(14.29)	54(85.71)	0.24(0.04-1.23)	0.730	0.32(0.04-2.23)	0.267	0.053
Alleles							
G	14(20.00)	56(80.00)	1		1		
A	26(14.94)	148(85.06)	0.73(0.37-1.44)	0.359	0.72(0.32-1.59)	0.420	0.084
rs4291							
Genotypes							
AA	11(21.57)	40(78.43)	1		1		
TA	04(6.78)	55(93.22)	**0.26(0.07-0.89)****	**0.003**	**0.23(0.06-0.85)***	**0.027**	**0.004**
TT	05(41.67)	07(58.33)	2.59(0.68-9.79)	0.158	3.65(0.86-15.48)	0.078	0.015
Alleles							
A	26(16.15)	135(83.85)	1		1		
T	14(18.82)	69(81.18)	1.20(0.61-2.39)	0.596	1.27(0.54-3.01)	0.571	0.114

**p-values <0.01; *p-values<0.05; CI: Confidence Interval; HPT=hypertension
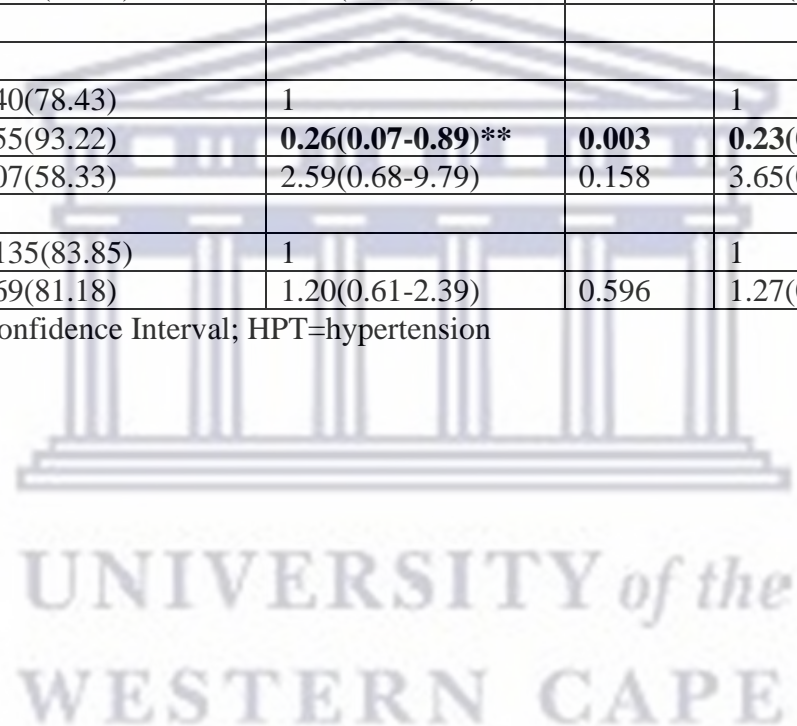
Discussion

Although, many advances have been made in the field of hypertension therapeutics, inter-individual variability in response to the various classes of anti-hypertensive drugs has been reported (13). However, there is a paucity of existing literature on the ethnically and genetically diverse population of South Africa in relation to pharmacogenomics-based anti-hypertensive therapy. In the current study, we describe single nucleotide polymorphisms (SNPs) in amlodipine associated genes and assesses their correlation with blood pressure control among South African adults with hypertension.

The current study examined twelve SNPs associated with amlodipine in 304 hypertensive individuals belonging to the indigenous Nguni tribe (Swati, Xhosa and Zulu) of South Africa. Out of twelve, only five SNPs (rs1042713, rs10494366, rs2239050, rs2246709 and rs4291) were detected among the Xhosa population. However, the variant alleles of these SNPs were not detected among South African Zulu and Swati tribal groups. The minor allele frequencies displayed by the Xhosa population were different from those observed in other African and global populations including the Luhya (Kenya), the Yoruba (Nigeria and Benin), African American (United States of America), British (Great Britain), South Asian and Mexican (California, USA) people (28). African populations particularly those located in Southern Africa are underrepresented in genomic studies (29) . The data presented in this study will help bridge the knowledge gap that exist, and possible contribute towards building an African-specific genomic database that could be utilized in personalised medicine. Furthermore, our study has highlighted the diversity that exist among indigenous black South Africans. These differences could be used in stratifying patient to responders and non-responder anti-hypertensive drugs including amlodipine. To the best our knowledge, this is the first study to

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detect and report all five SNPs in one of the indigenous tribes in South Africa. Given the wide variations of different tribal groups in South Africa, more studies are therefore recommended to further expand the frontiers of pharmacogenomics in the country.

The effect of *ADRB2* rs1042713 and *CYP3A5* rs2246706 on blood pressure response to amlodipine among hypertensive patients was investigated. No association between blood pressure response to amlodipine and the genotypes or the alleles of the two SNPs (rs1042713 and rs2246706) was established. In contrast, the G allele of rs1042713 (*ADRB2*) was significantly higher among Northern Han Chines individuals with essential hypertension (30). On the other hand, it was shown that blood pressure response to amlodipine among high-risk African-Americans appeared to be determined by CYP3A4 genotypes (17). The authors further demonstrated that hypertensive patients with the AA genotype of rs2246706 (CYP3A5) may have a decreased likelihood of reaching a target mean arterial pressure, in comparison to carriers of the AG and GG genotypes (17). The *CYP3A5* gene plays an important role in amlodipine metabolism, whilst the *ADRB2* gene encode for a primary adrenergic receptor that causes vasodilation in humans (31,32). Although both SNPs showed no association with blood pressure response to amlodipine in the current study cohort, previous findings suggest that both polymorphisms may be of relevance in amlodipine pharmacogenomics. Thus, the clinical use of both CYP3A4 and ADRB2 SNPs for personalized amlodipine treatment regimens should be further explored in larger hypertensive cohort of African origin.(31–33).

The present study also examined the association between NOS1AP rs10494366 and *CACNA1C* rs2239050 on blood pressure response to amlodipine among hypertensive patients. The genotypes and the alleles of NOS1AP rs10494366 and *CACNA1C* rs2239050 were not associated with blood pressure response to amlodipine. However, a recent study conducted among Caucasian patients receiving amlodipine treatment, showed that the GG genotype of rs2239050 was independently associated with improved treatment outcome rate of 52% (17).

170

Similar effects were observed among Chinese individuals (34). Also, the genotype GG was associated with improved blood pressure response to amlodipine among Caucasian patients who were also carriers of the GG genotype of *CACNA1C* rs2238032, suggesting a possible SNP-SNP interaction (11). The disparities observed in the present study and the reference studies may be due to different sample sizes as well as interference of other anti-hypertensive drugs that might have been prescribed to the patients. On the other hand, there is no record of the direct association of *NOS1AP* rs10494366 with blood pressure response to amlodipine. However, previous association studies suggest that this SNP may be associated with a higher risk of all causes of mortality among Caucasian participants on amlodipine therapy. It was further demonstrated that *NOS1AP* rs10494366 may be associated with a prolonged QTc interval in five independent populations (35). The *NOS1AP* is important in the pathophysiology of hypertension (12,13), whilst the *CACNA1* gene is the direct target of amlodipine (15). However, the clinical relevance of CACNA1C and NOS1AP SNPs with regards to amlodipine therapy among people of African origin remains unknown. Thus, more studies need to be conducted in order to establish the relationship between this SNP and blood pressure response to amlodipine among Africans and explore its possible use as a predictor of blood pressure response to anti-hypertensive drugs.

Xhosa carriers of the TA genotype of ACE rs4291 were less likely to exhibit controlled blood pressure in response to amlodipine therapy. There is no record of the direct association of this polymorphism with blood pressure response to Amlodipine in literature. However, the TA genotype was previously associated with decreased fasting plasma glucose levels among hypertensive patients undergoing amlodipine treatment (22). Also, rs4291 was associated with increased plasma ACE activity in endometrial cancer (21) This polymorphism occurs in a gene that is an important component the renin-angiotensin-aldosterone system (RAAS) which acts as a key regulator of electrolyte imbalance (36). As a result, the ACE gene is a good candidate

for studying the pathophysiology of uncomplicated hypertension and pharmacodynamics of metformin (9). If the concept of precision medicine is to be realised, the functional effect of rs4291 needs to be explored among a more diverse cohort that completely represent the people of South Africa.

Strength and limitations of the study

This is the first study to detect SNPs associated with amlodipine among indigenous South African tribes. In addition, this study reports an association between the TA genotype of rs4291 and blood pressure control with amlodipine among a South African cohort. However, some limitations of the study cannot be ignored; small sample size, lack of information on the dosing of anti-hypertensive drugs, and adherence to treatment and other lifestyle measures. These factors could have impacted on the extent of blood pressure control in the cohort. Also, the authors acknowledge the fewer samples of individuals on amlodipine monotherapy. This is largely due the standard of hypertension treatment in South Africa, where patients are initiated on thiazide diuretics upon diagnosis and amlodipine is introduced as an add-on drug for patients who do not respond adequately to thiazide monotherapy (37). Furthermore, there was no variation observed at the chosen loci among the Zulu and Swati tribes. Future studies with larger and representative sample of the Zulu and Swati population will build on the current study to further elucidate the future role of pharmacogenomics-based anti-hypertensive therapy. It should also be noted that this study purposively selected three populous tribal groups in the country; therefore, more studies are needed among other ethnic groups in South Africa.

Conclusion

This study reports the detection of five SNPs in amlodipine associated genes (rs2239050, rs2246709, rs4291, rs1042713 and rs10494366) among the indigenous Xhosa speaking tribe of South Africa. In addition, the TA genotype of rs4291 was associated with blood pressure

response to amlodipine treatment among the Xhosa cohort. Findings of the study highlight the relevance of comprehensively characterizing highly diverse populations, particularly those of African origin in order to facilitate pharmacogenomics-based anti-hypertensive treatment. Additionally, these findings might open doors for more pharmacogenomics studies, which could inform innovations to personalized anti-hypertensive treatment in the ethnically diverse population of South Africa.

Acknowledgements

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Chapter 6

Association of Five Single Nucleotide Polymorphisms with Enalapril Treatment Response among South African Adults with Hypertension

6.1 Introduction

The complex nature of hypertension and the physiological-regulating systems contributing to its progression and control, it is important to study polymorphisms directly or indirectly implicated in the pathways associated with the anti-hypertensive effect of pharmacological enalapril as well as other drugs in the class of ACE inhibitors. This will help better our understanding of the intricate physiology of drug response among hypertensive patients. This chapter examined the association of polymorphisms in the five selected genes with BP response to enalapril. The chapter further assessed genetic interactions that exist within these genes and their implications in enalapril treatment response among South African Adults with hypertension. Objective 4 is addressed by this manuscript.

6.2 Publication details

Title	Association of Five Single Nucleotide Polymorphisms with Enalapril Treatment Response among South African Adults with Hypertension
Authors	CM Masilela, B Pearce, JJ Ongole, OV Adeniyi and M Benjeddou
Authors' Contribution	CM, BP, JJO and MB conceptualised, designed and implemented the study protocol. CM and OVA analysed the data and drafted the manuscript. All authors revised and approved the final draft of the manuscript for submission
Ethics	BM/16/5/19
Journal	Medicine
Status	Awaiting publication Editors final decision

6.3 Journal information

Medicine® is an open access publication, providing authors with continuous publication of original research across a broad spectrum of medical scientific disciplines and sub-specialties. The Medicine® review process emphasizes the scientific, technical and ethical validity of submissions. Novelty or potential for impact are not considered during the manuscript's evaluation or adjudication.

6.4 Manuscript: Association of Five Single Nucleotide Polymorphisms with Enalapril Treatment Response among South African Adults with Hypertension

Abstract

Objective: This study investigates the association of five single nucleotide polymorphisms (SNPs) in selected genes (ABO, VEGFA, BDKRB2, NOS3 and ADRB2) with blood pressure response to enalapril. The study further assessed genetic interactions that exist within these genes and their implications in enalapril treatment response among South African adults with hypertension.

Methods: A total of 284 participants belonging to the Nguni tribe of South Africa on continuous treatment for hypertension were recruited. Five SNPs in enalapril pharmacogenes were selected and genotyped using MassArray. Uncontrolled hypertension was defined as blood pressure (BP) ≥140/90 mmHg. The association between genotypes, alleles and blood pressure response to treatment was determined by fitting multivariate logistic regression model analysis, and genetic interactions between SNPs were assessed by multi-factor dimensionality reduction (MDR). **Results:** Majority of the study participants were female (75.00%), Xhosa (78.87%) and had uncontrolled hypertension (69.37%). All five SNPs were exclusively expressed among Swati and Zulu participants. In the multivariate (adjusted) logistic model

analysis, ADRB2 rs1042714 GC [AOR=2.31; 95%CI 1.02-5.23; p=0.044], BDKRB2 rs1799722 CT [AOR=2.74; 95% CI 1.19-6.28; p=0.017] and C allele [AOR=0.37; 95%CI 0.15-0.94; p=0.037] were independently associated with controlled hypertension in response to enalapril. A significant interaction between rs699947, rs495828 and rs2070744 (CVC= 10/10; p=0.0005) in response to enalapril was observed.

Conclusions: We confirmed the association of rs1042714 (ADRB2) and rs1799722 (BDKRB2) with uncontrolled hypertension and established an interaction between rs699947 (VEGFA), rs495828 (ABO) and rs2070744 (NOS3) with BP response to enalapril. Our findings have provided substantial evidence for the use of SNPs as predictors for enalapril response among South Africans adults with hypertension.

Key words: Single nucleotide polymorphisms; Uncontrolled Hypertension; Enalapril**;** Gene-gene interaction, Pharmacogenomics

Introduction

The prevalence of hypertension has increased rapidly in the past decade, reaching epidemic proportions in the lower- and middle-income countries [1,2]. This increasing trend has also been reported in South Africa, especially among the predominant black ethnic group [3]. The age standardized prevalence of hypertension was estimated at 39.9% among urban dwelling Black South Africans [4]. Furthermore, the high burden of hypertension is attributed to the epidemiologic and nutritional transitions characterised by urbanisation and adoption of western habits such as unhealthy diets (excess salt and fat intake), reduced physical activity, increased alcohol consumption and tobacco use observed among Black South Africans [5]. Hypertension prevalence also mirrors the increasing trend of other non-communicable diseases (stroke, coronary heart diseases, peripheral artery diseases and heart failure) in the country.

179

Apart from lifestyle and environmental factors, genetic factors such as single nucleotide polymorphisms (SNPs) also play crucial roles in the occurrence of hypertension as well as blood pressure response to anti-hypertensive treatment [5–9]. However, there is currently insufficient evidence on the specific SNPs that predict blood pressure response to hypertension medication among the populations of African origin. Therefore, it is crucial to explore SNPs that may predict drug response in order to build an African-specific genetic profile that could be used in tailoring hypertension treatment for this population.

Enalapril, an angiotensin-converting enzyme (ACE) has played a pivotal role in the management of hypertension, heart failure, left ventricular dysfunction, and chronic kidney failure for many decades [10–12]. It exerts its blood pressure lowering properties by suppressing the formation of angiotensin II thereby reducing arterial pressure, preload and afterload on the heart [13]. The vasodilatory effect of enalapril is attributed to its ability to stimulate bradykinin B2 receptor on endothelial cells, which in turn promotes nitric oxide (NO) production [14,15]. In addition, it has been suggested that ACE inhibitors induce their vasodilatory effect by stimulating vascular endothelial growth factor (VEGF) levels through an interaction between BR2 and the angiotensin II type 2 receptor, thereby, promoting NO production through the stimulation of nitric oxide synthase (NOS3) in the vascular endothelial cells [16]. Like NOS3, beta-2 adrenergic receptor (ADRB2) is expressed in vascular endothelial cells. Furthermore, stimulation of ADRB2 activates adenylyl cyclase, which in turn induces cyclic adenosine-3′, 5′-monophospate. The latter triggers the NO system to activate vasodilatation by increasing arginine uptake [17].

Polymorphisms in genes coding proteins that are implicated in ACE inhibitor induced vasodilation have been associated with variable blood pressure in response to enalapril [14,16]. For instance, the rs1799722 polymorphism sits on the promoter region of Bradykinin receptor

B2 (*BDKRB2*) that encodes the BDKRB2 protein [16]. The rs699947 polymorphism is found on the Vascular Endothelial Growth Factor A (*VEGFA*) gene, which encodes the VEGF protein. On the other hand rs1042714 and rs2070744 are found on the beta-2 adrenergic receptor (*ADRB2*) gene and nitric oxide synthase 3 (*NOS3*) gene respectively [16,18]. The NOS3 gene encodes the enzyme NOS3, while *ADRB2* encodes the cell membrane-spanning ADRB2 protein [16,19,20]. Hypertensive carriers of the CA + AA genotypes for the rs699947 (*VEGFA*) and CT + CC genotypes of rs1799722 (*BDKRB2*) polymorphisms showed an increased response to enalapril treatment [14,16]. Moreover, GG+CG of rs1042714 (*ADRB2*) were associated with increased enalapril sensitivity among Europeans with left ventricular hypertrophy [21]. Whereas carriers of the TT genotype of 2070744 (*NOS3*) showed reduced response to enalapril treatment [16]. Candidate gene and haplotype analysis suggest that polymorphisms in genes implicated in vasodilation pathways may synergistically influence BP response to enalapril, suggesting a possible gene-gene interaction [20]. However, the individual as well as the synergistic effect of these polymorphisms in BP response to enalapril are yet to be established among Africans.

Histo-blood group ABO system transferase is an enzyme with glycosyltransferase activity, encoded by the ABO gene [22]. The gene was previously associated with variation in plasma ACE activity, inflammation, increased risk of hypertension and ACE inhibitor-induced cough [23–25]. While there is no record of the direct implication of ABO on the efficacy of ACE inhibitors, it is possible that polymorphisms in this gene may influence and predict BP in response to enalapril; owing to the recently established ABO-ACE plasma activity relationship.

Due to the multifactorial nature of hypertension and the complex physiological-regulating systems contributing to its severity and control, it is important to study polymorphisms directly or indirectly implicated in the pathways associated with the anti-hypertensive effect of

pharmacological drug. This will help advanced our understanding of the intricate physiology of drug response outcomes among hypertensive patients. Therefore, the current study examined the association of polymorphisms in the ABO, VEGFA, BDKRB2, NOS3 and ADRB2 genes with BP response to enalapril. Furthermore, the study assessed genetic interactions that exist within these genes and their implications in enalapril treatment response among South African Adults with hypertension.

Materials and methods

Ethical considerations

The Senate Research Committee of the University of the Western Cape approved the study protocol (Ethics approval number: BM/16/5/19). Permission to implement the study was granted by the clinical governance of the respective hospitals in the Eastern Cape and Mpumalanga Provinces. Consenting participants were issued with a research information sheet detailing the study in their home language. The rights to privacy and confidentiality of medical information of each participant were respected during and after the study.

Study design and patient selection

A total of 284 Nguni (Xhosa, Swati and Zulu) patients attending chronic care for hypertension were recruited consecutively between January 2019 and June 2019, from Cecilia Makiwane Hospital (East London, Eastern Cape), Piet Retief Hospital, Thandukukhanya Community Health Centre and Mkhondo Town Clinic (Mkhondo, Mpumalanga). Participants were eligible for participation if they were 18 years or older, and were on continuous treatment for hypertension for at least a year prior to the study. Individuals who were bedridden, pregnant and unable to give consent were excluded from the study.

Data collection

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Anthropometric measurements were conducted by a trained research nurse. The weight of each participant was measured to the nearest 0.1 kg using a digital scale (Tanita-HD 309, Creative Health Products, MI, USA) and height to the nearest of 0.1 cm using a mounted stadiometer, with participants wearing minimal clothing. The blood pressure of each participant was measured using a validated automated digital blood pressure monitor (Macrolife BP A 100 Plus model) according to standard protocols. Thereafter, BP was recorded in triplicate and the average was used to categorize participants into two groups: controlled (blood pressure <140/90 mmHg) and uncontrolled (blood pressure ≥140/90 mmHg) (26).

Age, ethnicity, smoking status and salt-intake were self-reported by each participant and documented in a proforma designed for this study. The number and type of anti-hypertensive drugs prescribed for each participant was retrieved from their clinical records. DNA samples were collected from each participant in the form of buccal swabs and stored at -20∘ C until they were extracted.

DNA isolation

Genomic DNA was extracted from buccal swab samples using a standard salt-lysis procedure [27]. Briefly, DNA samples were incubated in lysis buffer at 62 °C overnight. Thereafter, DNA was precipitated with NaCl followed by the addition of 75% ice-cold ethanol and incubated at -20 °C overnight. Precipitated DNA was purified using 70% ethanol and re-suspended in nuclease-free water. Samples were stored in 2 ml Eppendorf tubes at -20 °C until further use. DNA was quantified using a NanoDrop™ 2000/2000c Spectrophotometers (Thermo Scientific™) and Gel Doc™ EZ Gel Documentation System (BIO-RAD, USA).

Selection of SNPs and Genotyping

Five SNP previously associated with enalapril efficacy in individuals with hypertension were selected using Pharmacogenomics Knowledge Base [28], Ensembl [29] as well as an extensive survey of recent literature. Selected SNPs exhibited a PharmKGB evidence base of at least 3, indicating a variant-drug combination evaluated in multiple studies but lacking clear evidence of association.

Two multiplex MassARRAY systems (Agena BioscienceTM) were designed and optimized by Inqaba Biotechnical Industries (Pretoria, South Africa) in January 2017. Each multiplex was used to genotype selected SNPs, using an assay that is based on a locus-specific PCR reaction. This reaction is followed by a single base extension using the mass-modified dideoxynucleotide terminators of an oligonucleotide primer, which anneals upstream of the site of mutation. Matrix Assisted Laser Desorption/Ionization - time-of-flight (MALDI-TOF) mass spectrometry was used to identify the SNP of interest.

Statistical analysis

Statistical analyses were performed using IBM Statistical Package for Social Science (SPSS) Version 25 for Windows (IBM Corps, Armonk, New York, USA). The general characteristics of the participants were expressed as frequency count (percentages). The associations between alleles, genotypes and blood pressure response to enalapril were assessed by fitting multivariate logistic regression model analysis (unadjusted and adjusted odds ratios) and their 95% confidence intervals. Ethnicity, age, smoking status, alcohol consumption, physical activity, obesity, number of anti-hypertensive drugs prescribed as well as individual SNPs were used to adjust the final logistic regression model. Minor allele frequency (MAF) and Hardy-Weinberg equilibrium (HWE) tests were calculated using Genetic Analysis in Excel (GenAIEx) Version 6.5.

SNP-SNP interactions between ABO (rs495828), NOS3 (rs2070744), VEGFA (rs699947), BDKRB2 (rs1799722) were determined using Multi-factor dimensionality reduction (MDR) version 3.0.2. Only two genotypes (CC + GC) were expressed among carriers of ADRB2 (rs1042714) polymorphism, therefore; it was excluded from the analysis. The best model of interaction was selected on the basis of a high cross-validation consistency (CVC) score and p-values. P-values were calculated using x^2 test, values <0.05 were deemed significant.

Results

General characteristics of the study cohort

The study was comprised of 284 adults, of whom 213 (75.00%) were female and 71 (25.00%) were male. A total of 123 (43.31%) participants were Xhosa, 224 (78.87) had never smoked, 109 (38.38%) were aged ≥66 years, and 197 (69.37%) had uncontrolled blood pressure (≥140/90 mmHg) (Table 1).
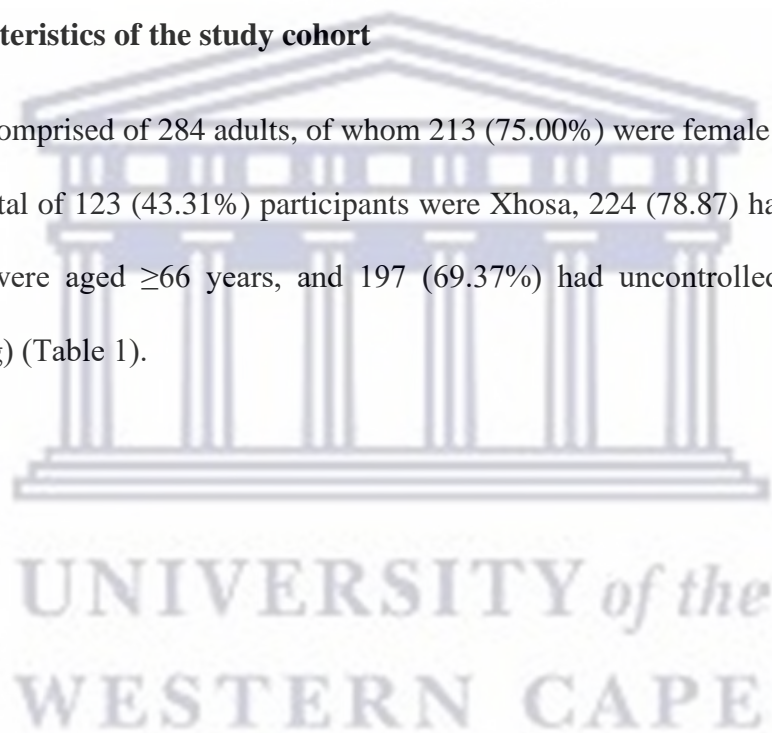
Table 1. Characteristics of the study participants (n=284)

Variables	Frequency (N=284)	Percentage (%)
Gender		
Male	71	25.00
Female	213	75.00
Age (Years)		
18 – 25	01	0.35
26 – 35	07	2.46
36 – 45	17	5.99
46 – 55	59	20.77
56 – 65	91	32.04
≥66	109	38.38
BMI		
None Obese	169	59.51
Obese	115	40.49
Ethnicity		
Zulu	120	42.25
Swati	41	14.44
Xhosa	123	43.31
Smoking status		
Never Smoked	224	78.87
Ever Smoked	60	21.13
Alcohol consumption		
Never Drank	222	78.17
Current Drinker	62	21.83
Physical Activity		
Active	69	24.29
Inactive	215	75.71
Salt intake		
Low-Moderate	235	82.75
Increased	49	17.25
Blood Pressure		
<140/90 mmHg	87	30.63
≥140/90 mmHg	197	69.37
Number of anti-hypertensive drugs		
Enalapril + 1	25	8.80
Enalapril + 2	69	24.29
Enalapril + 3	190	66.90

Expression patterns of SNPs across three population groups

The expression frequency of five SNPs was evaluated in three populations (Swati, Xhosa and Zulu). All five SNPs were exclusively expressed among Swati and Zulu participants. The variants rs1042714 (ADRB2) rs1799722 (BDKRB2) and rs495828 (ABO) showed an expression frequency of ≥75%. However; rs699947 (VEGFA) demonstrated an expression frequency of 72.5% among the Zulu population (Table 2). The Hardy–Weinberg equilibrium analysis was carried out for the study participants using the x^2-test.

All five SNPs were predominantly detected among participants who exhibited uncontrolled hypertension. On the other hand, rs1042714 and rs1799722 were detected among 48.78% and 53.38% of participants who were prescribed enalapril + 3 drugs, respectively. rs699947 was detected among 50.85% of participants who were prescribed enalapril + 3 drugs, whereas the same SNP was detected among 13.56% of participants who were prescribed enalapril + 1 drug. The expression patterns of the rest of the SNPs are shown in Table 3.

Table 2. Single nucleotide polymorphisms associated with Enalapril found in the participants disaggregated by ethnic groups, gender and age (n=284)

dbSNP	Gene	Ethnic Groups			Gender		Age		
		Zulu (n; %)	Swati (n; %)	Xhosa (n; %)	Male (n; %)	Female (n; %)	<55 Years	55-65 Years	>65 Years
All		120(42.25)	41(14.44)	123(43.30)	71(25.00)	213(75.00)	79(27.82)	95(33.45)	110(38.73)
rs1042714	*ADRB2*								
Yes		91(75.83)	32(78.05)	0(0.00)	21(29.58)	102(47.89)	33(41.77)	37(38.95)	53(48.18)
No		29(24.17)	09(21.95)	123(100)	50(70.42)	111(52.11)	46(58.23)	58(61.05)	57(51.82)
rs1799722	*BDKRB2*								
Yes		112(93.33)	39(95.12)	0(0.00)	25(35.21)	126(59.15)	46(58.23)	41(43.16)	64(58.18)
No		08(6.67)	02(4.88)	123(100)	46(64.79)	87(40.85)	33(41.77)	54(56.84)	46(41.82)
rs2070744	*NOS3*								
Yes		81(67.50)	33(80.49)	0(0.00)	18(25.35)	96(45.07)	29(36.71)	33(34.74)	52(47.27)
No		39(32.50)	08(19.51)	123(100)	53(74.65)	117(54.93)	50(63.29)	62(65.26)	58(52.73)
rs495828	*ABO*								
Yes		114(95.00)	39(95.12)	0(0.00)	24(33.81)	129(60.56)	46(58.23)	42(44.21)	65(59.09)
No		06(5.00)	02(4.87)	123(100)	47(66.19)	84(39.44)	33(41.11)	53(55.79)	45(40.91)
rs699947	*VEGFA*								
Yes		87(72.5)	31(75.60)	0(0.00)	20(28.17)	98(46.00)	33(41.11)	33(34.74)	52(47.27)
No		33(27.5)	10(24.39)	123(100)	51(71.83)	115(53.99)	46(58.23)	62(65.26)	58(52.73)

Table 3: Single nucleotide polymorphisms associated with Enalapril found in the participants disaggregated by BP control and anti-hypertensive drugs prescription patterns by Pearson chi square test

SNP	BP		p-value	Prescription			p-value
	Controlled (n; %)	Uncontrolled (n; %)		Enalapril+1 (n; %)	Enalapri+2 (n; %)	Enalapril+3 (n; %)	
rs1042714	51(41.46)	72(58.54)	0.275	17(13.82)	46(37.40)	60(48.78)	**0.174**
rs1799722	59(39.07)	92(60.93)	0.954	22(14.57)	50(33.11)	79(52.32)	0.743
rs2070744	46(40.35)	68(59.65)	0.621	15(13.16)	43(37.72)	56(49.12)	0.195
rs495828	57(37.25)	96(62.75)	**0.033**	21(13.72)	53(34.65)	79(51.63)	0.135
rs699947	54(45.76)	64(54.24)	**0.004**	16(13.56)	42(35.59)	60(50.85)	0.567

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Association of enalapril-associated SNPs with uncontrolled hypertension

In the multivariate logistic regression model (unadjusted) analysis, the GC genotype of rs1042714 was associated with controlled hypertension in response to enalapril treatment [OR=2.41(1.17-4.94); p=0.016]. No association was established between the genotypes or the alleles of rs1799722 (BDKRB2) rs495828 (ABO) and rs699947 (VEGFA) (Table 4).

In the adjusted model analysis, rs1042714 GC [AOR=2.31(1.02-5.23); p=0.044] and rs1799722 CT [AOR=2.74(1.19-6.28); p=0.017] were independently associated with controlled hypertension in response to enalapril. Furthermore, the C allele [AOR=0.37(0.15-0.94); p=0.037] was significantly associated with uncontrolled hypertension in response to enalapril (Table 4)

190
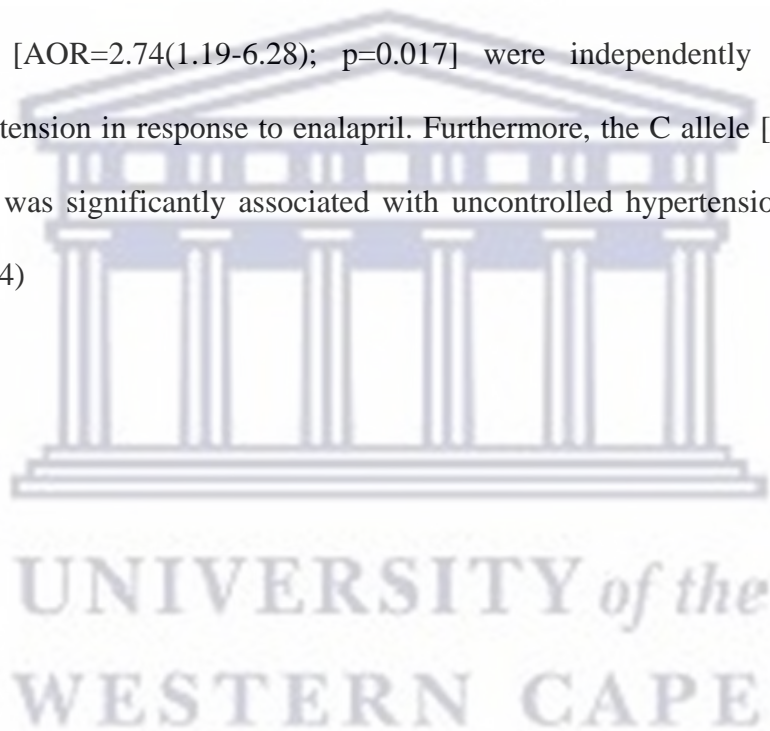
Table 4. Independent association of SNPs associated with Enalapril and Uncontrolled Hypertension (Multivariate logistic regression model)

DbSNP	Unadjusted odds ratios (95%CI)	p-value	Adjusted odds ratios (95% CI)	p-value
All				
rs1042714				
Genotypes				
GG			-	
CC	1		1	
GC	2.41(1.17-4.94)	0.016	2.31(1.02-5.23)	**0.044**
Alleles				
G	1		1	
C	0.84(0.37-1.72)	0.574	0.94(0.40-2.26)	0.903
rs1799722				
Genotypes				
CC	1		1	
TT	0.85(0.35-2.02)	0.729	0.97(0.37-2.56)	0.960
CT	1.90(0.94-4.02)	0.071	2.74(1.19-6.28)	**0.017**
Alleles				
C	1		1	
T	1.53(0.93-2.53)	0.093	1.31(0.72-2.38)	0.368
rs2070744				
Genotypes				
CC	1		1	
TT	0.60(0.25-1.47)	0.272	0.73(0.25-2.16)	0.582
TC	0.61(0.22-1.68)	0.346	0.76(0.22-2.56)	0.667
Alleles				
C	1		1	

T	0.73(0.33-1.61)	0.442	0.69(0.30-1.58)	0.386
rs495828				
GG	1		1	
TT	2.84(0.58-13.91)	0.197	2.10(0.36-12.10)	0.403
TG	1.40(0.50-3.19)	0.512	1.26(0.42-3.77)	0.479
Alleles				
G			1	
T	1.15(0.49-2.72)	0.734	0.86(0.32-2.29)	0.772
rs699947				
AA			1	
CC	0.93(0.33-2.59)	0.895	1.06(0.31-3.56)	0.923
CA	1.21(0.36-4.01)	0.750	1.79(0.44-7.18)	0.407
Alleles				
A	1		1	
C	0.43(0.17-1.03)	0.881	0.37(0.15-0.94)	**0.037**
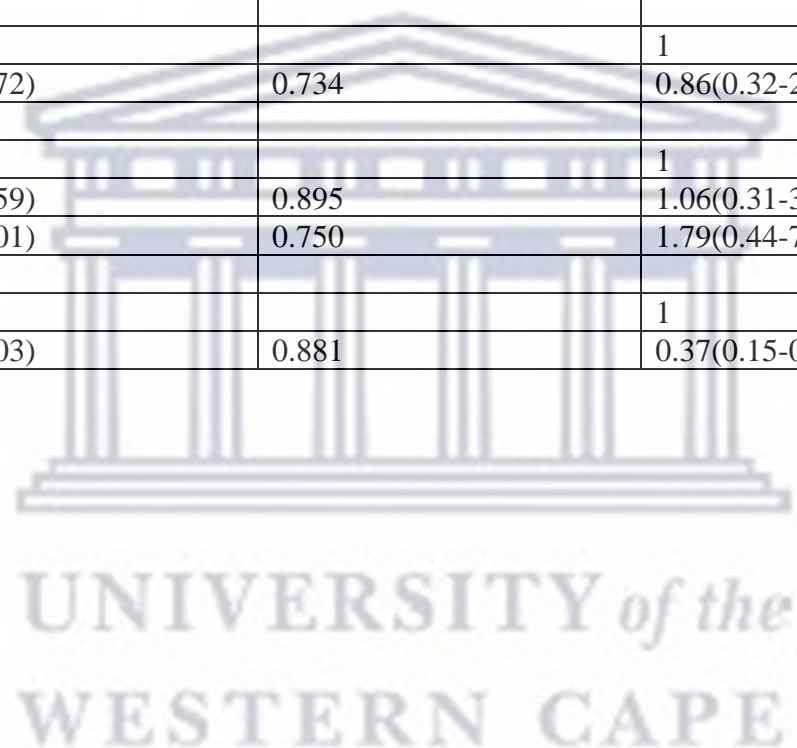
Interaction between enalapril-associated SNPs

Interactions among the ADRB2, ABO, NOS3, BDKRB2, VEGFA polymorphisms were assessed using Multifactor dimensionality reduction (MDR). The combination of rs699947 (VEGFA), rs495828 (ABO) and rs2070744 (NOS3) demonstrated a high CVC score (10/10) and was significantly (p= 0.0005) associated with blood pressure response to enalapril (Table 5).

Table 5: Interaction models among the VEGFA, NOS3 and ABO polymorphisms in hypertensive patients

Interaction Models	Training Score	Testing score	CVC	P-value
VEGFA_rs699947	0.5826	0.5826	10/10	0.3333
NOS3_rs2070744, VEGFA_rs699947	0.6356	0.5711	09/10	0.6251
NOS3_rs2070744, ABO_ rs495828, VEGFA_ rs699947	0.6776	0.5518	10/10	**0.0005**

194

Additionally, the combination of CC (rs699947), TT (rs2070744) and GG (rs495828) were expressed more frequently among participants with controlled hypertension. Whereas GG (rs495828), CT (rs2070744) and CC (rs699947) was expressed more frequently among participants with uncontrolled hypertension (Figure 1).
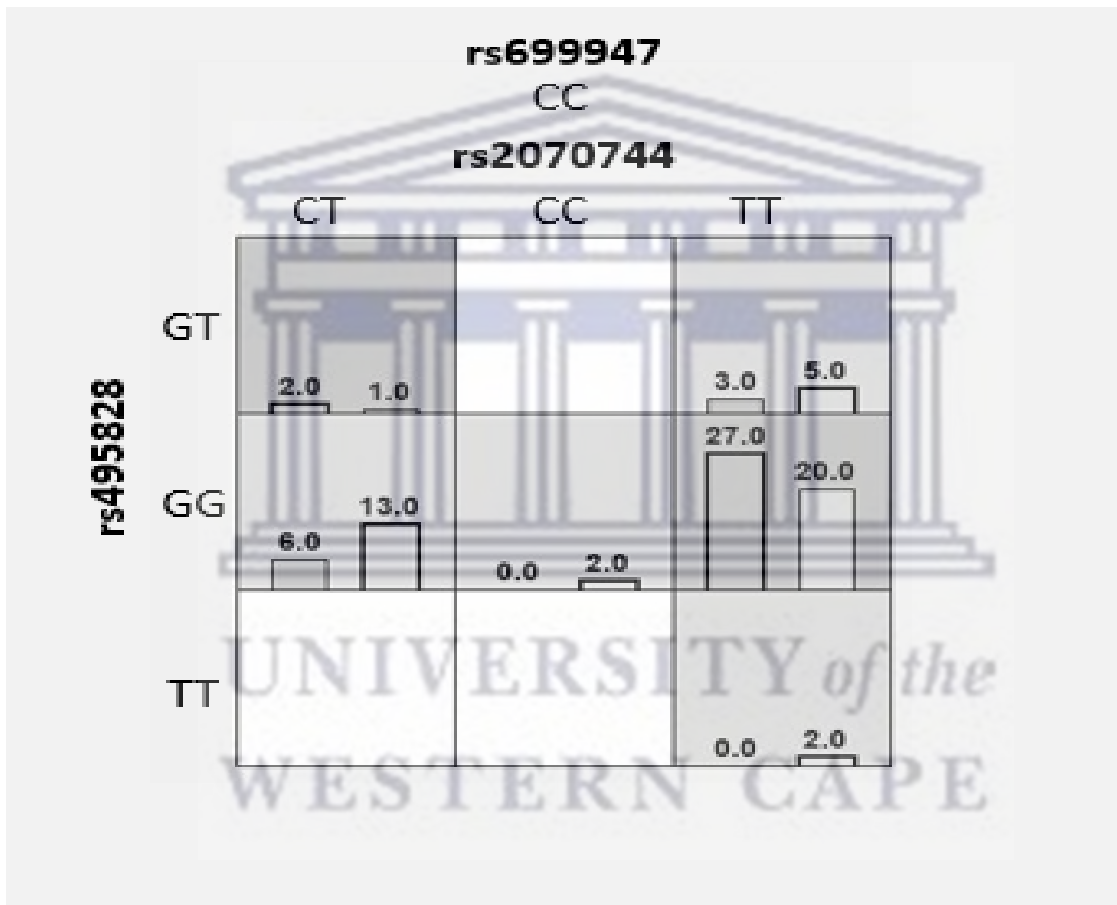

Figure 1: The best MDR model of interaction among ABO rs495828, NOS3 rs2070744 and VEGFA rs699947. The distributions of Controlled (left bars) and Uncontrolled (right bars) are illustrated for each combination of genotypes. Each cell represents genotype combinations. Dark grey cells represent genotype combinations implicated in enalapril treatment outcome.

Discussion

There is substantial variation in blood pressure response to anti-hypertensive therapy brought by existing genetic factors [7,30]. However, genetic factors associated with enalapril treatment outcome are understudied in Black Africans. Therefore, the present study examined the possible association of five SNPs with blood pressure response to enalapril therapy. The study further assessed genetic interaction patterns between ABO (rs495828), NOS3 (rs2070744), VEGFA (rs699947), BDKRB2 (rs1799722) and ADRB2 (rs1042714) and their implication in blood pressure response to enalapril among South African adults with hypertension.

South African Nguni people are classified into four sub-groups, Swati, Ndebele, Xhosa and Zulu. Together, they represent two-thirds of South Africa's Black population [31]. The four groups are culturally, linguistically, and genetically diverse, however; under-represented in previous drug association studies [32]. It is possible that the four groups present SNP expression patterns that are distinct from each other, that may possibly predict their response to hypertensive drugs including enalapril. The current study cohort comprised of 284 hypertensive patients, of whom 120 (42.25%) were Zulu, 41(14.44%) were Swati and 123(43.30) were Xhosa. The five SNPs genotyped, were exclusively expressed among the Swati and Zulu participants. Although, the Ndebele group was not represented and the Swati group was under-represented, the findings of this study highlight the genetic differences that exist within the Nguni population, which may be a result of major events that took place within South Africa such as migration and admixture.

Beta 2-Adrenergic receptors are up-regulated in hypertension and largely polymorphic within the human population [21]. In addition, the ADRB2 gene has been widely researched as a candidate gene for essential hypertension and anti-hypertensive drug response due to its role in vasodilatation [19,21]. The current study investigated the association of rs1042714 (ADRB2) with BP response

196

to enalapril in patients with hypertension. The GC genotype of rs1042714 was independently associated with controlled hypertension in response to enalapril. Furthermore, the genotype GC was previously associated with an increased response to enalapril among Caucasian patients with left ventricular hypertrophy [21]. The study further demonstrated that the GG genotype was associated with a similar effect. However, a randomized trial that was conducted among African Americans demonstrated that the genotypes of rs1042714 have no effects on BP response to ramipril [19]. Ramipril falls under ACE inhibitors along with enalapril and they are assumed to influence blood pressure in a similar pattern [33]. Although the effect of rs1042714 was not assessed in the current study cohort, it appears that the anti-hypertensive effect of rs1042714 is exclusive to enalapril.

Vascular endothelial growth factor is an important angiogenic factor encoded by the VEGFA gene [34]. Furthermore, VEGF stimulates endogenous NO resulting in vasodilation [14]. Owing to its role in blood pressure regulation, it is expected that polymorphisms in this gene may influence BP response to ACE inhibitors including enalapril. In the present study, the effect of rs699947 was assessed on BP response to enalapril. The C allele of rs699947 was significantly associated with uncontrolled hypertension. While there is no record of the association of the C allele with decreased response to enalapril, Brazilian carriers of the CC and AA genotypes showed decrease in mean BP following treatment with 20 mg/day enalapril [14]. Haplotype analysis of rs699947, rs1570360 and rs2010963 demonstrated that carriers of the AGG haplotype had a better blood pressure response following enalapril treatment. Whereas carriers of the CGG haplotype showed an opposite effect [14]. Taking these results into consideration, it is possible that the BP lowering effect of rs6999947 is highly dependent on the A allele. Also, the effect of enalapril on BP may be dependent on the interaction of rs699947 with rare variants other than rs1570360 and rs2010963.

197

Bradykinin is a potent vasodilator that plays an important role in the pathophysiology of hypertension [35]. Also, polymorphisms in the gene that mediated the production of bradykinin are implicated in enalapril treatment outcome [16,20]. In this study, the CT genotype of the promoter variant rs1799722 was significantly associated with controlled hypertension in response to enalapril treatment. The CT genotype was previously associated with decreased BP among patients undergoing enalapril therapy in comparison to the TT genotype [16]. The authors further demonstrated that CT genotype of NOS3 rs2070744 in combination with CC genotype of rs1799722 was frequently expressed among patients who exhibited an increased response to enalapril treatment. While the combination of CC (rs1799722) and TT (rs2070744) was expressed more frequently among patients who exhibited poor response to enalapril treatment [16]. In the present study, there was no association established between the genotypes of rs2070744 and BP response to enalapril. However, it appears that the effect of rs1799722 on BP response to enalapril may in part depend on the presence of NOS3 rs2070744. Studies investigating the association of rs2070744 and blood pressure response in a large African cohort need to be conducted, in order to assess its suitability as a marker for enalapril response.

Epistatic interactions are likely to play a pivotal role in the pathophysiology of hypertension as well as therapeutic interventions [6]. Epistatic interactions between rs2070744 (NOS3) and rs1799722 (BDKRB2) associated with BP response to enalapril were reported by Silva et al (2012). Also, Oliveira-Paula et al (2016) reported a significant interaction between rs16960228 (PRKCA) rs2070744 (NOS3) and rs1799722 (BDKRB2) associated with the anti-hypertensive effect of enalapril. This study investigated the interaction between ABO (rs495828), NOS3 (rs2070744), VEGFA (rs699947), and BDKRB2 (rs1799722) in enalapril response. An interaction between rs699947 (VEGFA), rs495828 (ABO) and rs2070744 (NOS3) was observed.

198

Furthermore, the genotype combination of CC (rs699947), TT (rs2070744) and GG (rs495828) were expressed more frequently among participants with controlled hypertension, while the combination of GG (rs495828), CT (rs2070744) and CC (rs699947) was expressed more frequently among participants with uncontrolled hypertension. To date, epistatic interaction patterns that have been established and associations with BP in response to enalapril have only included SNPs in genes that are directly implicated in vasodilation [16,20]. Single nucleotide polymorphism in genes that are not directly implicated in this pathway, such as ABO rs495828, have been left out despite their association with enalapril treatment outcome as well as the pathophysiology of hypertension [23,35]. Furthermore, this study found no association between the genotypes of rs495828 (ABO) with BP response to enalapril. We also found no record of the association of this SNP with BP in response to enalapril. However, our results suggest that rs495828 may influence BP in response to enalapril depending on the genotypes of rs2070744 and rs699947. These findings warrant future investigation on the direction of association of the individual genotypes of rs495828, and the underlying mechanism that links the ABO gene with the anti-hypertensive effect of enalapril.

Study limitations

Given the cross-sectional nature of the design of the study, causal association cannot be inferred. It should also be noted that this study included participants who were on enalapril in combination with other anti-hypertensive medications. This is supported by the South African hypertension practice guideline [36], which recommends thiazide diuretic or calcium channel blockers as first line treatment. As such, it was difficult to find patients on enalapril monotherapy, therefore, we could not avoid interference from other anti-hypertensive drugs. Although limiting, this strategy allowed us to collect and genotype a reasonable number of samples for an association study.

Furthermore, the ARDB2 polymorphism rs1042714 only expressed two genotypes, as a result it was excluded from the MDR interaction model.

Conclusion

This study reports the association of the genotypes rs1042714 (GC) and rs1799722 (CT) with BP response to enalapril treatment among South African adults. An association between the C allele of rs699947 and uncontrolled hypertension in response to enalapril treatment was also reported. For the first time in an indigenous South African cohort, an interaction between ABO (rs495828), NOS3 (rs2070744) and VEGFA (rs699947) associated with enalapril treatment outcome was established, suggesting that these SNPs may synergistically influence BP response to enalapril. These finding have provided substantial evidence for the use of polymorphism in genes directly implicated in ACE inhibitor pathways as predictors for enalapril response among hypertensive patients. Furthermore, this study has laid a foundation that will lead to the better understanding of gene-gene interactions within the ACE inhibitor pathway.

Declarations

Ethical approval and consent to participate

The study protocol was approved by the Research Ethics Committee of the University of the Western Cape. The Mpumalanga Department of Health and Piet Retief hospital clinical governance gave permission for the implementation of the study protocol across the three sites. The objectives of the study were explained and written informed consent was obtained from each participant. The research process followed the Helsinki Declaration and the rights of individuals to privacy and confidentiality were respected throughout the period of the study. Participation in the study was voluntary and no compensation was offered to any of the participants.

Consent to publish

Not applicable for this paper.

Availability of data and materials

All the study materials and data are available from the corresponding author, upon reasonable request.

Competing interests

The authors declare no conflict of interest.

Authors' Contributions

CM, BP, JJO and MB conceptualised, designed and implemented the study protocol. CM and OVA analysed the data and drafted the manuscript. All authors revised and approved the final draft of the manuscript for submission.

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Chapter 7

Factors Associated with Glycaemic Control among South African Adult Residents of Mkhondo Municipality

7.1 Introduction

The influencing factors of glycaemic control in individuals with DM in rural settings is poorly understood. Therefore, investigating the factors that are associated with poor glycaemic control is important in order to guide the crafting of context-specific interventions toward improving the clinical outcomes of people with DM in rural South Africa. This chapter bridges the missing gaps by describing the socio-demographic and clinical profiles of individuals with DM and determines the rate and influencing factors of glycaemic control among adult residents of Mkhondo Municipality in South Africa. Objective 5 is addressed by this manuscript.

7.2 Publication details

Title	Factors Associated with Glycaemic Control among South African Adult Residents of Mkhondo Municipality
Authors	CM Masilela, B Pearce, JJ Ongole, OV Adeniyi and M Benjeddou
Authors' Contribution	CM, BP, JJO and MB conceptualised, designed and implemented the study protocol. CM and OVA analysed the data and drafted the manuscript. All authors revised and approved the final draft of the manuscript for submission
Ethics	BM/16/5/19
Journal	Medicine
Status	Published (doi: 10.1097/MD.0000000000023467)

7.3 Journal information

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7.4 Manuscript: Factors Associated with Glycaemic Control among South African Adult Residents of Mkhondo Municipality

Abstract

Objectives: This study examines the rate and the influencing factors of glycaemic control among adult residents living with DM in Mkhondo Municipality of South Africa. **Methods:** In this cross-sectional study, 157 individuals attending care for DM were recruited. Glycaemic control status was categorized as poor if glycated haemoglobin (HbA1c) > 7% and very poor if HbA1c ≥ 9%. Multivariate regression analysis was used to identify the significant determinants of poor and very poor glycaemic control. **Results**: The majority of the study participants were females (84.71%) and above 45 years old (88.55%). The overall prevalence of poor glycaemic control was 77.71% (n=122), whilst very poor glycaemic control occurred in 50.6% (n=80) of the study cohort. In the multivariate logistic regression model analysis, African traditional (AOR=0.15; 95%CI 0.04-0.57), fast food consumption (AOR=5.89; 95%CI 2.09-16.81), elevated TC (OR=2.33; 95%CI 1.50-5.17), elevated LDL-C (AOR=5.28; 95%CI 1.89-14.69) and TG (AOR=4.39; 95%CI 1.48-13.00) were the independent and significant determinants of poor glycaemic control. Age (AOR=0.46; 95%CI 0.23-0.92) was the only independent and significant determinant of very poor glycaemic control. **Conclusion**: We found a high rate of poor glycaemic control (77.71%) possibly attributed to religious affiliation, fast food consumption and dyslipidemia. On the other hand, about half of the study sample had very poor glycaemic control (HbA1c ≥ 9%) which was predominant among younger cohort

with diabetes mellitus. Interventions aimed at improving glycaemic control in this population must also target religious practice, dietary patterns and dyslipidaemia as well as tailored-approach for young people.

Keywords: Diabetes Mellitus; Mkhondo Municipality; Uncontrolled diabetes; South Africa

Introduction

The prevalence of diabetes mellitus (DM) in sub-Saharan Africa is increasing at an alarming rate and South Africa is at the forefront of the epidemic [1]. Currently, the overall prevalence of type 2 diabetes in South Africa is estimated at 12.8%, which differs by geographical settings [2]. While some studies reported high prevalence of 26.6 to 60% among adult residents in urban settings [3–5], others reported figures as low as 7.6% in the rural and semi-rural areas of the country [6]. Diabetes has a major impact on the lives of individuals, families and public health [7]. As such, South Africa has committed to lowering the burden of the disease in line with the National Development Plan [8]. However, the magnitude and the speed in which diabetes has evolved in this country calls for emergency intervention.

Diabetes is defined as a complex metabolic disease that is characterized by chronic hyperglycaemia [9,10]. Although the pathophysiology of type 2 diabetes is not completely understood, impaired insulin secretion and increased insulin resistance, which may be a result of an interplay between environmental and genetic factors jointly, contribute to the development and progression of the disease [11,12]. Over time, chronic hyperglycaemia may lead to long-term damage and failure of various organs, progressive development of specific complications such as retinopathy, nephropathy, stroke and cardiovascular diseases [13,14].

Given the complex etiology of type 2 diabetes, its treatment and management require a multi-pronged approach that enables patients to achieve and maintain near normal glycaeted hemoglobin (HbA1c) levels [15]. Studies have shown that achieving and maintaining normal

levels of HbA1c are crucial in the prevention of microvascular complications, cardiovascular events and associated morbidity and mortality [15,16]. Compelling evidence suggests that achieving and maintain the recommended glycaemic levels requires the use of both oral and injectable anti-diabetic therapy [15]. Furthermore, it has been suggested that combination therapy, with metformin and insulin, notably improves glucose control, lower the incidence of cardiovascular diseases and minimize insulin requirements among patients with DM [17]. When initiated early, combination therapy has demonstrated a long-term durability in comparison to any form of monotherapy [18]. However, poor adherence to medications due to side effects, complications, frequent dosing, polypharmacy and lack of education on diabetes self-management present a great challenge in the management of DM [19,20]. In addition, a negligent healthcare system that fails to intensify therapy appropriately when treatment goals have not been met may be a major contributor to poor glycaemic control among patients [21].

Diabetes is typically a life-long disease with incidence of death increasing steeply with duration of the disease [21]. Also, patients who have been living with diabetes for a longer duration demonstrate an earlier onset of diabetes-related complications and tend to require intense pharmacological and non-pharmacological interventions [22]. Therefore, it is unclear if the glycaemic control status of individuals with DM differ by the duration of diagnosis and types of treatment modalities, especially among adult residents of rural communities of South Africa. More so, the influencing factors of glycaemic control in individuals with DM in the rural Mkhondo municipality is poorly understood. These findings are needed to guide the crafting of context-specific interventions toward improving the clinical outcomes of people with DM in the region. The present study bridges the missing gaps by describing the socio-demographic and clinical profiles of individuals with DM, determines the rate and influencing factors of glycaemic control among adult residents of Mkhondo Municipality in South Africa.

Methods

208

This cross-sectional study was conducted across three primary health care centres in the rural Mkhondo Municipality of Mpumalanga Province, South Africa. Mkhondo is a small resource-constrained border town situated between the Kingdom of Eswatini and the KwaZulu-Natal province of South Africa. The municipality is made up of three township and three government health facilities serving a combined population of 189,036 residents.

A total of 157 individuals attending chronic care for DM were recruited consecutively between January 2019 to June 2019. A sample size of 157 was estimated by using the formula for cross sectional study: $\{N = (Z1 - \alpha) \, 2 \times P \, (1 - P) \, / \, D2\}$. Participants were eligible if they were at least 18 years old, had been on treatment for DM for a year and had been attending regular follow-up visits at any of the three study sites. Pregnant and clinically unstable patients were excluded from the study.

Participants underwent face-to-face interviews using a standardized questionnaire which comprised three major items, namely, demographic, lifestyle behaviours and clinical data. The interviews were conducted by a trained research nurse who also performed anthropometric measurements (weight and height) according to standard protocols. The body mass index (BMI) of each participant was estimated and categorized as obese if BMI ≥ 30.0kg/m^2 or not. Clinical data were extracted from the medical records of each participant. In addition, fasting venous blood samples for lipid assays and glycated haemoglobin were drawn by the research nurse. All blood assays [HbA1c, total cholesterol (TC), low-density lipoprotein (LDL-C), triglyceride (TG) and high-density lipoprotein HDL-C)] were conducted by the National Health Laboratory Services (NHLS) in accordance with standardized protocols.

Poor glycaemic control was defined as glycated haemoglobin (HbA1c) > 7% in accordance with the guidelines of the Society for Endocrinology, Metabolism and Diabetes of South Africa

(SEMDSA, 2017). In addition, participants with HbA1c ≥ 9% were further categorized as having very poor glycaemic control.

Complete data for 157 participants were captured and analyzed by using the Statistical Package for Social Science (SPSS) version 25 for Windows (SPSS Inc., Chicago, IL, USA). The socio-demographic and clinical characteristics of the participants were expressed as mean ± standard deviation for continuous variables and frequency (percentages) for categorical variables. The associations between the demographic, lifestyle behaviours and glycaemic control were examined at different cut-offs; first at HbA1c > 7% (poor glycaemic control) versus good glycaemic control (HbA1c ≤7%), followed by HbA1c ≥ 9% (very poor glycaemic control) versus fair glycaemic control (HbA1c < 9%) by using a chi-square test. Multivariate odd ratios (crude and adjusted), using logistic regression model analysis, were estimated with their 95% confidence intervals (95% CI) to identify the independent and significant determinants of poor and very poor glycaemic control. A p-value < 0.05 was considered for statistical significance.

Results

The majority of the participants were female (84.71%), above 45 years old (88.55%), Zulu-speaking (82.80%), practicing Christians (87.26%), employed (68.78%), had attained post-primary education (75.16%), never smoked cigarettes (86.62%) nor consumed alcohol drink (77.07%), consumed fruits and vegetables weekly (98.09%), consumed fast food weekly (67.52%) and engaged in a sedentary lifestyle (65.61%) (Table 1).

Table 1. Demographic characteristics of the study participants

Variables	Frequency (n)	Percentage (%)
Sex		
Male	24	15.29
Female	133	84.71
Age (Years)		
18 – 25	03	1.91
26 – 35	08	5.09
36 – 45	07	4.45
46 – 55	40	25.47
56 – 65	52	33.12
≥66	37	23.56
Ethnicity		
Zulu	130	82.80
Swati	27	17.20
Religion		
Christianity	137	87.26
African Traditional	20	12.74
Employment status		
Employed	108	68.78
Unemployed	49	31.21
Educational Level		
Primary	39	24.84
Post Primary	118	75.16
Smoking status		
Never Smoked	136	86.62
Ever Smoked	21	13.38
Alcohol consumption		
Never Drank	121	77.07
Occasional	36	22.93
Fruit and Vegetable Consumption		
1-3 times/week	154	98.09
Never	03	1.91
Fast Food Consumption		
Never	51	32.48
1-3 times/week	106	67.52
Physical Activity		
Active	54	34.39
Inactive	103	65.61

211
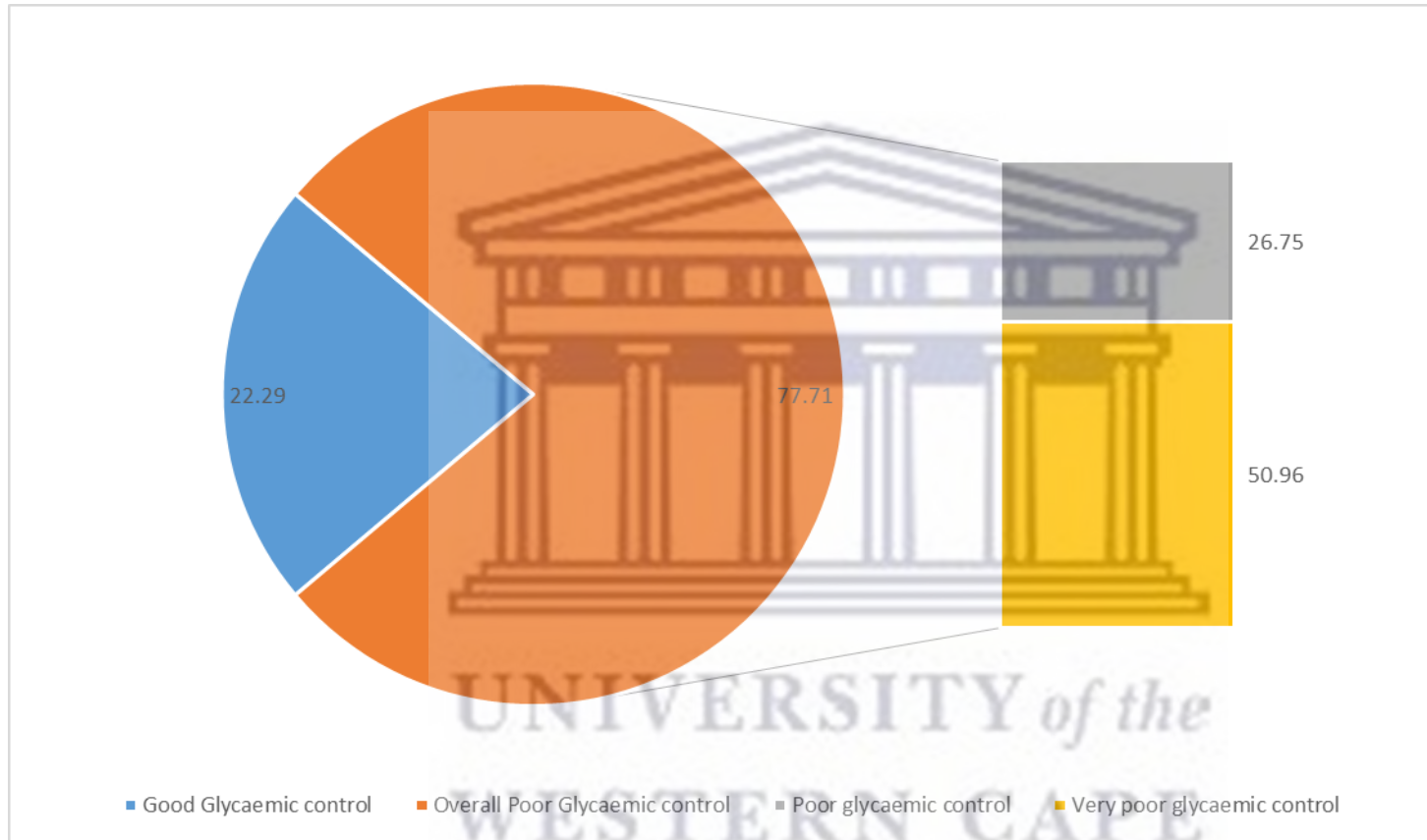
Figure 1: Overall poor glycaemic control status of the participants

Table 2: Chi-square test showing associations between glycaemic control and socio-demographic and clinical factors

Variables	Good Glycaemic control	Poor Glycaemic control	*p-value*	Fair Glycaemic Control	Very Poor Glycaemic Control	*p-value*
	≤7% HbA1C	**>7 HbA1c**		**<9 HbA1c**	**≥9 HbA1c**	
Gender			0.472			0.586
Male	04(16.67)	20(88.33)		13(54.17)	11 (45.83)	
Female	31(23.31)	102(76.69)		64(48.12)	69 (51.88)	
Age (Years)			0.080			**0.026**
<55 years	10(15.38)	55(84.62)		25(38.46)	40(61.54)	
≥55 years	25(27.17)	67(72.83)		53(56.99)	40(43.01)	
Employment status			0.426			0.748
Employed	26(24.07)	82(75.93)		53(44.17)	67(55.83)	
Unemployed	09(18.37)	40(81.63)		24(64.86)	13(35.14)	
Educational Level			0.142			0.991
Primary	12(30.77)	27(69.23)		22(56.41)	17(43.59)	
Post Primary	23(19.49)	95(80.51)		55(46.61)	63(53.39)	
Religion			**0.001**			0.289
Christianity	25(18.25)	112(81.75)		63(46.32)	73(53.68)	
African Traditional	10(50.00)	10(50.00)		17(70.83)	07(29.17)	
Fruit and Vegetable Consumption			0.643			0.529
1-3 times/week	34(22.08)	120(77.92)		75(48.70)	79(51.30)	
Never	01(33.33)	02(66.67)		02(66.67)	01(33.33)	
Fast Food Consumption			**0.007**			0.538
Never	18(35.29)	33(64.71)		30(58.82)	21(41.18)	
1-3 times/week	17(16.04)	89(83.96)		47(44.34)	59(55.66)	
Physical Activity			0.775			0.089
Active	15(23.43)	49(76.56)		27(42.19)	37(57.81)	
Inactive	20(21.50)	73(78.49)		50(53.76)	43(46.24)	

213

Total Cholesterol			**0.035**			0.135
<4.5 mmol/L	24(28.92)	59(71.08)		44(53.01)	39(46.99)	
≥4.5 mmo/L	11(14.86)	63(85.14)		33(44.59)	41(55.41)	
HDL-C			0.356			0.292
≥1.2 mmol/L	17(19.54)	70(80.46)		38(43.68)	49(56.32)	
<1.2 mmol/L	18(25.71)	52(74.29)		39(55.71)	31(44.29)	
LDL-C			**0.000**			0.134
<1.8 mmol/L	27(37.50)	45(62.50)		40(55.56)	32(44.44)	
≥1.8 mmo/L	08(9.41)	77(90.59)		37(43.53)	48(56.47)	
Triglycerides			**0.000**			0.133
<1.7 mmol/L	20(40.00)	30(60.00)		28(56.00)	22(44.00)	
≥1.7 mmol/L	15(13.64)	95(86.36)		48(45.28)	58(54.72)	
Duration of Diagnosis			0.573			0.068
< 5 years	28(23.33)	92(76.67)		61(50.83)	59(49.17)	
≥5 years	07(18.92)	30(81.08)		16(43.24)	21(56.76	
Treatment Regime			0.126			0.419
Oral	34(29.94)	108(76.06)		72(50.70)	70(49.30)	
Insulin/ Insulin + Oral	01(6.67)	14(93.33)		05(33.33)	10(66.67)	
Hypertension			0.379			0.201
No	07(29.17)	17(70.83)		14(58.33)	10(41.67)	
Yes	28(21.05)	105(78.95)		63(47.37)	70(52.63)	
Obesity			0.809			0.323
No	11(21.15)	41(78.85)		23(44.23)	29(55.77)	
Yes	24(22.86)	81(77.14)		54(51.43)	51(48.57)	

HDL-C=High density lipoprotein cholesterol; LDL-C=Low density lipoprotein cholesterol

214

Overall, the majority of the participants had poor glycaemic control (77.71%) and about half of the participants (n=80) had very poor glycaemic control (Fig. 1). The rate of poor and very poor glycaemic control differed by socio-demographic and clinical characteristics (Table 2). In chi-square analysis, there was a significantly higher risk of poor glycaemic control in individuals who were Christians, consumed fast food, had elevated TC, elevated LDL-C and elevated TG. Beside age, all other participants' characteristics were not significantly associated with the risk of having very poor glycaemic control.

In the multivariate (crude) logistic regression model analysis (Table 3), African traditional religion, consumption of fast food, elevated TC, elevated LDL-C and elevated TG were the independent and significant determinants of poor glycaemic control. Similarly, after adjusting for other covariates (Table 3), the magnitude and direction of association remained for African traditional religion, consumption of fast food and LDL-C; however, TC became insignificant while the direction of association changed for TG. Patients who were practicing African traditional religion were less likely to have poor glycaemic control compared to those practicing Christianity. However, patients with elevated LDL-C were five times more likely to have poor glycaemic control compared to those with normal LDL-C. Similarly, patients with elevated TG were four times more likely to have poor glycaemic control compared to those with normal TG.

Table 3: Adjusted and unadjusted logistic regression models showing socio-demographic and clinical factors associated with poor glycaemic control glycemic control (HbA1C>7%)

Variables	Unadjusted odds ratios (95% CI)	Adjusted odds ratios (95% CI)
All		
Gender		
Male	1	1
Female	1.50(0.48-4.78)	2.12(0.51-8.89)
Age (years)		
<55 years	1	1
≥55 years	0.48(0.22-1.10)	0.75(0.27-2.13)
Ethnicity		
Zulu	1	1
Swati	1.32(0.46-3.78)	1.30(0.31-5.39)
Employment Status		
Employed	1	1
Unemployed	1.40(0.60-3.28)	1.07(0.35-3.16)
Religion		
Christianity	1	1
African Traditional	**0.22(0.84-0.59)****	**0.15(0.04-0.57)****
Fast Food Consumption		
Never	1	1
1-3 times/week	**2.85(1.31-6.19)****	**5.89(2.09-16.81)****
Total Cholesterol		
<4.5 mmol/L	1	1
≥4.5 mmol/L	**2.33(1.50-5.17)****	1.24(0.39-3.23)
LDL-C		
<1.8 mmol/L	1	1
≥1.8 mmol/L	**5.77(2.41-13.79)*****	**5.28(1.89-14.68)****
Triglycerides		
<1.7 mmol/L	1	1
≥1.7mmo/L	**0.25(2.41-13.79)*****	**4.39(1.48-13.00)****
Duration of Diagnosis		
<5 years	1	1
≥5 years	1.30(0.57-3.29)	1.05(0.34-3.19)
Treatment Regime		
Oral	1	1
Insulin/Oral + Insulin	4.40(0.55-34.75)	0.60(0.59-7.00)

**p-values <0.01; ***p-values<0.001; CI: Confidence Interval; HDL-C=High density lipoprotein cholesterol; LDL-C=Low density lipoprotein cholesterol

In the multivariate (crude and adjusted) logistic regression model analysis (Table 4), very poor glycaemic control (HbA1c ≥ 9%) was compared with fair glycaemic control (HbA1c < 9%), it was also found that only the age of the participants was significantly associated with the risk of having very poor glycaemic control. Older patients (≥55 years) were less likely to have very poor glycaemic control in comparison to the younger individuals.

Table 4: Adjusted and unadjusted logistic regression models showing socio-demographic and clinical factors associated with very poor glycaemic control (HbA1C ≥9%)

Variables	Unadjusted odds ratios (95% CI)	Adjusted odds ratios (95% CI)
All		
Gender		
Male	1	1
Female	0.78(0.32-1.87)	0.70(0.27-1.78)
Age (years)		
<55	1	1
≥55	**0.48(0.25-0.91)***	**0.46(0.23-0.92**)*
Ethnicity		
Zulu	1	1
Swati	0.87(0.38-2.00)	0.73(0.28-1.89)
Employment Status		
Employed	1	1
Unemployed	1.0(0.51-1.97)	0.76(0.36-1.58)
Duration of Diagnosis		
<5 years	1	1
≥5 years	1.35(0.64-2.85)	1.28(0.59-2.27)
Treatment Regime		
Oral	1	1
Insulin/Oral + Insulin	2.05(0.66-6.32)	2.33(0.66-8.24)

*p-values<0.05; CI: Confidence Interval; HDL-C=High density lipoprotein cholesterol; LDL-C=Low density lipoprotein cholesterol

Discussion

In the current study, we examined factors influencing glycaemic control in individuals with DM in the rural Mkhondo municipality, South Africa. This is largely a rural, resource constrained setting and an understudied region of the country. The overall prevalence of poor glycemic control was 77.71%. This finding is worse than previous reports from South Africa [3–6] where the prevalence of poor glycaemic controlled ranged from 7.6%-60.0%; however, it is better than the 82.35% [23] and 83.8% [24] reported in the rural and semi-urban communities in the Eastern Cape, South Africa. In comparison to other studies conducted in other African countries, the rate reported in this study is better than previous reports from Ghana (86.4%) [25] and Sudan (85.0%) (26). Unfortunately, it is worse than rates reported in Ethiopia (70.8%) [27] and Kenya (60.5%) (28). The high prevalence of poor glycaemic control may be due to low awareness of the disease as well as sub-optimal treatment that is often observed among rural dwelling populations [23 – 26]. Our findings highlight the need to intensify glycaemic control in individuals with DM, given the life-threatening and economic impacts of the disease.

There is an overwhelming amount of evidence with regards to how religion and spirituality influence glycemic control across different populations [27, 29]. Some studies suggest that most spiritual individuals who are living with DM present poor self-care in comparison to their non-spiritual counterparts [27]. However, the extent to which the African traditional religion influences glycaemic control among South Africans is poorly understood. Our findings suggest that affiliation with the African traditional religion is associated with poor glycaemic control (HbA1c >7%). Although our study highlights the need to consider and address religious

practices of patients in the care for DM, further studies are recommended so as to gain a better insight into the effect of religious practices on glycaemic control.

Medical nutrition therapy is an important aspect of diabetes management. As such, diets rich in sugar, refined carbohydrates and high in fat have been associated with the incidence of diabetes [30,31]. In the current study, weekly consumption of fast food was associated with poor glycaemic response. These findings corroborate previous observations made in the United Arab Emirates, where fast food consumption was an independent predictor of poor glycemic control in patients with DM [32]. The study further demonstrated that consuming fresh fruits could have a protective effect on glycaemic control among patients. Although fruit and vegetables consumption were investigated in the present study, no association was established with poor glycaemic control. Future studies with a larger cohort of patients with DM might provide more insight into the association between glycaemic status and consumption of fruits and vegetables.

Diabetic dyslipidemia, characterized by high plasma triglycerides, high LDL-cholesterol and low HDL-cholesterol, is associated with poor glycemic control and cardiovascular risk [33]. As such, tight glycaemic control in patients with DM may lead to an improved lipid profile and a reduction in cardiovascular disease risk [33,34]. It was also demonstrated that aggressive therapy, that includes statin and lifestyle interventions aimed primarily at lowering LDL-C, do improve glycaemic control among patients with DM [35]. In the current study, increased TC, LDL-C and triglycerides were associated with poor glycaemic control. However, we do not know whether the study population was initiated on statin therapy. As a result, the lipid lowering effect of statins was not evaluated.

Insulin resistance and beta cell dysfunction are the most prominent metabolic features of type 2 diabetes [9]. Moreover, early initiation of insulin, alone or in combination with metformin,

has been shown to improve glycaemic control and preserve pancreatic β-beta cell function in patients with DM [15,17]. In the present study, no significant effect on glycaemic control was observed among patients receiving insulin alone or in combination with metformin. The lack of association observed may be a result of both the patient and clinician's reluctance to initiate insulin therapy because of perceived safety issues such as weight gain and hypoglycaemia [36]. Also, poor compliance with treatment by patients and lack of potency of insulin as a result of improper storage may have contributed to the lack of effect observed.

In the current study, age (<55 years) was associated with very poor glycaemic control (HbA1c ≥9%). It is plausible that younger patients may not yet internalize the chronicity of the disease and by implication, adjust to the necessary lifestyle changes including compliance with clinic visits and adherence to medications. Though, diabetes mellitus is considered a disease of the elderly; however, studies conducted in China [37] and the United States of America showed that sub-optimal glycaemic control was more common in younger adults [35], due to poor adherence to medication and clinic appointments [38]. Hence, our finding is consistent with the previous reports. Given the unique characteristics of DM among older adults, previous studies have shown that achieving good glycaemic control in older adults requires a tailored therapeutic approach that will eliminate the risk of cardiovascular disease and hypoglycaemia.

Limitations

Given the small sample size and the cross-sectional design of the study, the identified determinants should not be considered as causation. Notwithstanding, the small sample included, this study was conducted in three primary health care centres serving the predominant rural communities of Mkhondo Municipality. As such, the findings are generalisable to the population of individuals living with DM in the region and similar settings in the country. This

is the first study to report the rate of glycaemic control and its influencing factors in the rural Mkhondo Municipality of South Africa.

Conclusion

We found a high rate of poor glycaemic control (77.71%) possibly attributed to religious affiliation, fast food consumption and dyslipidemia. On the other hand, about half of the study sample had very poor glycaemic control (HbA1c ≥ 9%) which was predominant among younger cohort with diabetes mellitus. Interventions aimed at improving glycaemic control in this population must also target religious practice, dietary patterns and dyslipidaemia as well as tailored-approach for young people. These findings would guide the local authorities and clinicians in crafting and implementing appropriate interventions to improve the clinical outcomes in people with DM in the region.

Abbreviations

DM=diabetes mellitus; HDL-C=high density lipoprotein cholesterol; LDL-C=low density lipoprotein cholesterol; NHLS=National Health Laboratory Services; SEMDSA=Society of Endocrinology, Metabolism and Diabetes of South Africa; TC=total cholesterol; TG=triglyceride

Declarations

Ethical approval and consent to participate

The study protocol was approved by the Research Ethics Committee of the University of the Western Cape. The Mpumalanga Department of Health and Piet Retief hospital clinical governance gave permission for the implementation of the study protocol across the three sites. The objectives of the study were explained and written informed consent was obtained from each participant. The research process followed the Helsinki Declaration and the rights of individuals to privacy and confidentiality were respected throughout the period of the study. Participation in the study was voluntary and no compensation was offered to any of the participants.

Consent to publish

Not applicable for this paper.

Availability of data and materials

All the study materials and data are available from the corresponding author, upon reasonable request.

Competing interests

The authors declare no conflict of interest.

Authors'   Contributions

CM, BP, JJO and MB conceptualised, designed and implemented the study protocol. CM and OVA analysed the data and drafted the manuscript. All authors revised and approved the final draft of the manuscript for submission.

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Chapter 8

Single Nucleotide Polymorphisms Associated with Metformin and Sulphonylureas' Glycaemic Response among South African Adults with Type 2 Diabetes Mellitus

8.1 Introduction

This chapter address objective 6. It is highly evident that a more personalised approach may be beneficial in the management of T2DM. As such, there is an urgent need to investigate polymorphisms that define response to metformin/SU combination therapy among people of African ancestry. The manuscript presented in this chapter examined the association of nine polymorphisms belonging to SLC22A1, SP1, PRPF31, NBEA, SCNN1B, CPA6 and CAPN10 genes with glycaemic response to metformin/SU combination therapy among South African adults with T2DM. In addition, the study further assesses the epistatic interactions between these SNPs and their response to metformin/SU combination therapy.

8.2 Publication details

Title	Single Nucleotide Polymorphisms Associated with Metformin and Sulphonylureas' Glycaemic Response among South African Adults with Type 2 Diabetes Mellitus
Authors	CM Masilela, B Pearce, JJ Ongole, OV Adeniyi and M Benjeddou
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8.3 Journal information

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8.4 Manuscript: Single Nucleotide Polymorphisms Associated with Metformin and Sulphonylureas' Glycaemic Response among South African Adults with Type 2 Diabetes Mellitus

Abstract

Aims: To examine the association of polymorphisms belonging to *SLC22A1, SP1, PRPF31, NBEA, SCNN1B, CPA6* and *CAPN10* genes with glycaemic response to metformin and sulfonylureas (SU) combination therapy among South African adults with diabetes mellitus type 2 (T2DM).

Methods: A total of 128 individuals of Swati (n=22) and Zulu (n=106) origin attending chronic care for T2DM were recruited. Nine SNPs previously associated with metformin and SUs were selected and genotyped using MassArray. Uncontrolled T2DM was defined as HbA1c>7%. The association between genotypes, alleles and glycaemic response to treatment was determined using multivariate logistic regression model analysis.

Results: About 85.93% (n=110) of the study participants were female and 77.34% (n= 99) had uncontrolled T2DM (HbA1c >7%). In the multivariate (adjusted) logistic regression model analysis, the CC genotype of rs2162145 (*CPA6*), GG and GA genotypes of rs889299 (*SCNN1B*) were significantly associated with uncontrolled T2DM. On the other hand, the C allele of rs254271 (*PRPF31*) and the GA genotype of rs3792269 (CAPN10) were associated with controlled T2DM. A significant interaction between rs2162145 and rs889299 in response to metformin and SU combination therapy was observed.

Conclusions: In this study, we reported the association of rs2162145 (CC), rs889299 (GG and GA) with uncontrolled T2DM. We also reported the association of rs254271 (C) and rs3792269 (GA) with controlled T2DM in response to metformin and SU combination therapy.

228

Furthermore, an interaction between rs2162145 and rs889299 was established, where the genotype combination GA (rs889299) and TT (rs2162145) was associated with uncontrolled T2DM.

Keywords: Type 2 diabetes; Single nucleotide polymorphisms; Metformin; Sulfonylureas; Combination therapy.

Introduction

Diabetes mellitus (DM) is a chronic non-communicable disease that affects about 463 million people world-wide [1]. By the year 2030, the burden of DM is expected to increase by 10.2% reaching an estimated 578 million cases globally [1]. According to the International Diabetes Federation (IDF), over 4.5 million South African adults were estimated to be living with DM in the year 2019. Furthermore, over two million of these individuals were undiagnosed and were at a higher risk for life-threatening complications associated with DM [1]. Using data from the South African National Health and Nutrition Survey (2011-2012), Stokes et al. showed that 18.1% of South Africans were treated, but exhibited poor glycaemic control (HbA1C>7%) [2]. Uncontrolled DM and its complications have a huge and rapidly growing impact on the South African health care system [1–3]. Thus, improving glycaemic control in individuals diagnosed with DM and initiated on treatment will require concerted efforts from many fronts including individual behavioural changes, public health efforts and tailored medical care that is guided by pharmacogenomics strategies.

Diabetes Mellitus, particularly type 2 diabetes (T2DM) is a complex metabolic disease that is characterized by hyperglyceamia as a consequence of defects in insulin secretion, insulin action or a combination of both [4]. To date, there are more than ten classes of drugs that are used to manage T2DM [5,6]. However, metformin is the only approved anti-diabetic drug under the class of biguanides that is indicated for the treatment of T2DM [7]. Metformin exerts its anti-

diabetic properties by decreasing hepatic glucose production, decreasing intestinal absorption of glucose and increasing insulin sensitivity. Thus, improving glucose uptake and utilization in peripheral tissues [8,9]. On these grounds, metformin is the preferred initial oral anti-diabetic agent [10]. However, when monotherapy fails to achieve glycaemic goals, combination therapy using a second agent with a different mechanism of action is often initiated [6,11,12]. Sulfonylureas (SUs) are characterised as insulin secretagogues, as they stimulate insulin secretion in the pancreatic beta-cells. This class of drugs may also improve peripheral and hepatic insulin sensitivity by reducing glucose toxicity [12–14]. The most commonly prescribed SUs are glibenclamide, gliclazide and glimepiride [15,16]. These three drugs are classified as second-generation SUs and they are the preferred add-on agents to metformin therapy [6]. Despite these efforts, treatment with any class of anti-diabetic drug features variability that is brought by Single Nucleotide Polymorphisms (SNPs) in genes that are directly or indirectly implicated in the pharmacokinetics and pharmacodynamics of anti-diabetic agents [9].

Specificity protein 1 (SP1) is a zinc finger transcription factor that binds to GC-rich motifs of many promoters including those found in the solute carrier gene superfamily that is responsible for metformin transport [17]. Calpain 10 (*CAPN10*) is calcium-dependent cysteine proteases that is implicated in glucose metabolism and pancreatic beta-cell function [18]. Owing to their respective roles in metformin transport and glucose metabolism, SNPs in both genes were associated with insulin resistance, T2DM and response to anti-diabetic drugs [19,20]. For instance, the G allele of rs3792269 (*CAPN10*) was associated with an absolute reduction of HbA1c following a six-month treatment with metformin among Caucasian patients with T2DM [21]. On the other hand, rs2683511 (*SP1*) was associated with decreased metformin secretory clearance among a mixed American cohort, however, this effect was demonstrated among healthy individuals [22].

230

Carboxypeptidase A6 (CPA6) is an enzyme that is encoded by the *CPA6* gene. The enzyme is responsible for catalyzing the release of C-terminal amino acids and have functions ranging from digestion to selective biosynthesis of neuroendocrine peptides [23]. On the other hand, pre-mRNA processing factor 31(PRPF31) encodes a ubiquitously expressed mRNA splicing factor [24]. Furthermore, Rotroff et al. (2018) demonstrated that rs254271 (*PRPF31*) was associated with decreased metformin response among patients of European and African origin. Whilst rs2162145 of *CPA6* was associated with better response to metformin in the same study cohort, it was further demonstrated that rs57081354, an intronic polymorphism found in the Neurobeachin (*NBEA*) gene was associated with a decreased metformin response [25] . While the role of these genes in the pharmacokinetics and pharmacodynamics of metformin is unknown, recently published data suggest that polymorphisms situated in these genes may predict metformin response in individuals with T2DM [25].

In addition, solute carrier family 22 member 1 (*SLC22A1*) is poly-specific organic cation transporters encoded by the *SLC22A1* gene that plays an important role in the influx of metformin in hepatocytes and its elimination through the renal system [26]. A number of *SLC22A1* SNPs have been associated with variable metformin response in individuals with T2DM [27]. For instance, it has been shown that European carriers of the del/del genotype of rs36056065 (*SLC22A1*) may have a decreased but not absent risk of gastrointestinal side effects associated with metformin [28]. On the other hand, carriers of the del/del genotype of rs72552763 (*SLC22A1*) exhibited decreased hepatic distribution and exposure to metformin in healthy individuals residing in Denmark [26]. Moreover, Lebanese carriers of the AC and AA genotype of rs622342 (*SLC22A1*) showed a greater HbA1c reduction following metformin/SU combination therapy [29].

It was further demonstrated that rs889299 was associated with T2DM and that European carriers of the AA genotype may have an increased risk of oedema when treated with

glibenclamide [30]. The rs889299 polymorphism occurs in Sodium Channel Epithelial 1 Subunit Beta (*SCNN1B*), a gene that encodes for the beta-subunit of epithelial sodium channel (*ENaC*) [30]. Literature suggest that ENaC activity may be regulated by ATP-binding cassette protein such as the K channel-associated sulfonylurea receptor [31]. Glibenclamide is a known inhibitor of the K channel-associated sulfonylurea receptor. Furthermore, the drug increased transepithelial Na transport *in vitro* [31]. On these grounds, it is possible that rs889299 may influence glycaemic response in individuals with T2DM undergoing SU monotherapy or metformin/SU combination therapy. Also, it is possible that epistatic interactions between this variant and co-existing polymorphism may influence glycaemic response to metformin/SU combination therapy. This effect is yet to be established in patients with T2DM of African descent.

It is becoming increasingly evident that a more personalised approach may be beneficial in the management of T2DM. Given the dearth of pharmacogenomics researches among individuals of African ancestry, data generated from other population groups are unlikely to reflect the overall effect of SNPs in anti-diabetic response among Africans. As such, there is an urgent need to investigate this identified gap with specific focus on polymorphisms that define response to metformin/SU combination therapy in African population. This study examines the association of nine polymorphisms belonging to *SLC22A1, SP1, PRPF31, NBEA, SCNN1B, CPA6* and *CAPN10* genes with glycaemic response to metformin/SU combination therapy among South African adults with T2DM. In addition, the study further assesses the epistatic interactions between these SNPs and their response to metformin/SU combination therapy.

Materials and Methods

Study design and patient selection

Ethical clearance for this study was obtained from the Senate Research Committee of the University of the Western Cape (Ethics clearance number BM/16/5/19). Permission to implement the study was granted by the clinical governance of Piet Retief Hospital and the department of Health of the Mpumalanga Provinces. All participants received information on the purpose and procedure of the study in the home language (Swati and Zulu) prior to signing an informed consent by each participant.

A total of 128 individuals of Swati (n=22) and Zulu (n=106) origin attending chronic care for T2DM were recruited consecutively between January 2019 and June 2019, from the outpatient department of Piet Retief Hospital, Thandukukhanya Community Health Center and Mkhondo Town Clinic (Mkhondo, Mpumalanga). The study included participants who were 18 years or older, and were on continuous Metformin/SU dual therapy for T2DM for at least a year prior to the study. Patients who were pregnant, diagnosed with Type 1 diabetes mellitus, malignancies, chronic kidney and liver disease as well as those who were undergoing monotherapy of either insulin, metformin or any other drug for T2DM were excluded.

Data collection

Anthropometric measurements were conducted by a trained research nurse. The weight of each participant was measured to the nearest 0.1 kg using a digital scale (Tanita-HD 309, Creative Health Products, MI, USA) and height to the nearest of 0.1 cm using a mounted stadiometer, with participants wearing minimal clothing. Body Mass Index (BMI) for each patient was estimated as weight (kg) divided by height (m^2). We further categorised BMI as: underweight=BMI<18.5kg/m^2; normal weight=BMI: 18.5-24.9kg/m^2; overweight=BMI: 25.0-29.9kg/m^2; obese=BMI ≥30 kg/m^2. Blood assays for glycated haemoglobin (HbA1c) were conducted by the National Health Laboratory Services (NHLS) in accordance with

standardized protocols. Uncontrolled T2DM was defined as HbA1c >7% in accordance with the guidelines of the Society for Endocrinology, Metabolism and Diabetes of South Africa.

Duration of T2DM and anti-diabetic drugs prescribed for each participant were retrieved from their clinical records. Prescribed SUs were glibenclamide, glimepiride and gliclazide in combination with metformin. Age, ethnicity and physical activity were self-reported and documented in a proforma designed for this study. Physical activity was classified into active if participants engaged in rigorous physical activity that increased heart rate, and inactive if participants did not take part in any form of physical activity. Ethnicity was defined as belonging to a social group with a common language, cultural tradition or ancestry; Swati or Zulu. DNA samples were collected from each participant in the form of buccal swabs and stored at -20∘ C until they were extracted.

DNA isolation

Genomic DNA was isolated from buccal swabs using a standard salt lysis method (32). Extracted DNA samples were stored at -20°C. DNA was quantified using a NanoDrop™2000/2000c UV/VIS Spectrophotometer (ThermoScientific™). SNPs were genotyped using the MassARRAY®System IPLEX extension reaction (Agena Bioscience™). Genotypes of the selected SNP variants were determined for all the study participants.

Selection of SNPs and Genotyping

Nine SNPs previously associated with Metformin or Sulfonylurea treatment outcome were selected using Pharmacogenomics Knowledge Base [33], Ensembl [34] as well as an extensive survey of recent literature. Two multiplex MassARRAY systems (Agena Bioscience TM) were designed and optimized by Inqaba Biotechnical Industries (Pretoria, South Africa) in January 2017. Each multiplex was used to genotype selected SNPs, using an assay that is based on a locus-specific PCR reaction. This reaction is followed by a single base extension using the

mass-modified dideoxynucleotide terminators of an oligonucleotide primer, which anneals upstream of the site of mutation. Matrix Assisted Laser Desorption/Ionization - time-of-flight (MALDI-TOF) mass spectrometry was used to identify the SNP of interest.

Statistical analysis

Statistical analyses were performed using IBM Statistical Package for Social Science (SPSS) Version 25 for Windows (IBM Corps, Armonk, New York, USA). The general characteristics of the participants were expressed as frequency (percentages). The associations between alleles, genotypes and glycaemic response to metformin/SU combination therapy were assessed by multivariate logistic regression model analysis (unadjusted and adjusted odds ratios) and their 95% confidence intervals. The final model of the adjusted logistic regression analysis included rs2162145, rs2282143, rs254271, rs2683511, rs3792269, rs57081354, rs72552763, rs36056065 and rs622342. Results for the unadjusted logistic regression model analysis were expressed as crude odds ratios (CORs) and adjusted odds ratios (AORs) for the adjusted logistic regression model analysis. A p-value less than 0.05 was considered statistically significant. Bonferroni corrected p-values were set at < 0.0125. The Minor allele frequency (MAF) and Hardy-Weinberg equilibrium (HWE) tests were calculated using Genetic Analysis in Excel (GenAIEx) Version 6.5. SNP-SNP interactions between rs5708135, rs2162145, rs36056065, rs622342 and rs889299 were determined using Multi-factor dimensionality reduction (MDR) version 3.0.2. Sp1 rs2683511 (TT), rs3792269 (GG) and rs72552763 (del/del) were not detected, therefore; they were excluded from the analysis. The best model of interaction was selected on the basis of a high cross-validation consistency (CVC) score and p-values. P-values were calculated using x^2 test, values <0.05 were deemed significant.

Results

General characteristics of the study cohort

A total of 128 individuals with T2DM undergoing metformin/SU combination therapy were recruited. About 14.06% (n=18) were male, and 85.93% (n=110) were female, of whom 35.93% (n=46) were aged between 55-65 years. Furthermore, the cohort was comprised of 82.81% (n=106) and 17.19% (n=22) individuals of Zulu and Swati origin, respectively. Majority of the study participants (68.75%) were obese (BMI $\geq$30kg/m^2), 60.93% (n=78) were inactive, 77.34% (n= 99) had uncontrolled T2DM (HbA1c > 7%) and 73.44% (n=94) have been living with T2D for < 5 years (Table 1).

Table 1: General characteristics of the study cohort

Variable	Total (n; %)	Male (n; %)	Female (n, %)
	128(100%)	18(14.06)	110(85.93)
Ethnicity			
Swati	22(17.19)	04(22.22)	18(16.36)
Zulu	106(82.81)	14(77.78)	92(83.64)
Age			
18-25 years	02(1.56)	01(5.55)	01(0.91)
26-35 years	02(1.56)	0(0.00)	02(1.81)
36-45 years	15(11.72)	02(1.56)	13(11.81)
46-55 years	31(24.22)	05(27.77)	26(23.63)
56-65 years	46(35.93)	05(27.77)	41(37.27)
$\geq$65 year	32(25.00)	05(27.77)	27(24.54)
BMI			
<18.5 kg/m^2	01(0.78)	0(0.00)	01(0.91)
18.5-24.9 kg/m^2	13(10.16)	04(22.22)	09(8.18)
25.0-29.9 kg/m^2	26(20.31)	06(4.69)	20(18.18)
$\geq$30 kg/m^2	88(68.75)	08(4.44)	80(72.72)
T2DM treatment outcome			
HbA1C $\leq$7%	29(22.66)	06(33.33)	45(40.90)
HbA1C >7 %	99(77.34)	12(66.67)	65(59.09)
Duration of Diagnosis			
<5 years	94(73.44)	13(72.22)	81(73.64)
$\geq$5 years	34(26.56)	05(27.78)	29(26.36)
Physical Activity			
Active	50(39.06)	08(44.44)	42(38.18)
Inactive	78(60.93)	10(55.56)	68(61.82)

Expression and association of SNPs with metformin/SU combination therapy response

Seven (rs2162145, rs2282143, rs254271, rs2683511, rs3792269, rs57081354, rs72552763) out of nine SNPs were within Hardy-Weinberg equilibrium (HWE) with p-values ranging from 0.134-0.771 (Table 2).

Table 2: **SNP information and Hardy-Weinberg p-values for each SNP in the study cohort**

SNP	Gene	Chromosomal location	Location	Amino acid change	Drug	HWE p-value
rs2162145	*CPA6*	8:67747912	Intergenic	T>C	Metformin	0.771
rs2683511	*Sp1*	12:53410706	Intron	C>T	Metformin	0.670
rs3792269	*CAPN10*	2:240592062	Synonymous	A>G	Metformin	0.313
rs254271	*PRPF31*	19:54127382	Intron	G>C	Metformin	0.134
rs57081354	*NBEA*	13:35202457	Intron	C>T	Metformin	0.955
rs36056065	*SLC22A1*		Intron	GTAAGTTG > del	Metformin	0.002
rs622342	*SLC22A1*	6:160151834	Intron	A>C	Metformin	0.021
rs72552763	*SLC22A1*	6:160139849	Inframe Deletion	GAT>del	Metformin	0.522
rs889299	*SCNN1B*	16:23370593	Intron	A>G	Glibenclamide	0.504

HWE= Hardy Weinberg equilibrium

In the multivariate logistic regression (unadjusted) model analysis, the CC of rs622342 (COR= 4.65; 95%CI 1.08-19.86; p=0.038), rs889299 (COR=3.55; 95%CI 1.11-11.33; p=0.032) and C allele of rs57081354 (COR=2.15; 95%CI 1.14-4.04; p=0.017) were significantly associated with uncontrolled DM, whilst the G allele of rs3792269 (COR=0.33; 95%CI 0.12-0.88; p=0.027) was associated with controlled DM (Table 3). No association was observed between uncontrolled DM and the genotypes or alleles of rs2162145, rs2683511, rs36056065, rs72552763 and rs254271 (p>0.05).

After adjusting with each SNP, the multivariate logistic regression (adjusted) model analysis showed that the CC genotype rs2162145 (*CPA6)* (AOR=14.86; 95%CI 1.71-29.04; p=0.014), GG (AOR=7.91; 95%CI 1.67-37.27; p=0.009) and GA (AOR=5.27; 95%CI 1.19-23.19; p=0.028) genotypes of rs889299 (*SCNN1B*) were significantly associated with uncontrolled T2DM. On the other hand, the C allele of rs254271 (AOR=0.20; 95%CI 0.07-0.62; p=0.005) and the GA genotype of rs3792269 (*CAPN10*) (AOR=0.15; 95%CI 0.02-0.85; p=0.033) were associated with controlled T2DM (Table 3). Polymorphisms rs2683511, rs57081354, rs36056065 and rs622342, rs72552763 were not associated with uncontrolled DM in response to Metformin/SU combination therapy (P>0.05). The Bonferroni correction p-value was set at < 0.0125. After Bonferroni correction, the rs3792269 (GA), rs254271(C), rs2162145 (CC) and rs889299 (GG and GA) remained significant with p-values < 0.0125.

Table 3: Association of SNPs with glycaemic response to metformin/SU combination therapy

dbSNP	Unadjusted odds ratios (95% CI)	p-Value	Adjusted odds ratios (95% CI)	p-Value	Bonferroni Adjusted p-Values
All					
rs2162145					
Genotypes					
TT	1		1		
CT	2.21(0.45_10.71)	**0.323**	6.67(0.99-46.22)	0.051	
CC	4.95(0.84-29.00)	0.076	14.86(1.71-129.04)	**0.014**	**0.0035**
Alleles					
T	1		1		
C	0.84(0.42-1.65)	0.615	0.77(0.37-1.61)	0.500	
rs254271					
Genotypes					
GG	1		1		
GC	0.29(0.35-2.44)	0.257	0.10(0.01-1.28)	0.077	
CC	0.27(0.03-2.36)	0.738	0.13(0.01-1.16)	0.119	
Alleles					
G	1		1		
C	1.34(0.55-3.24)	0.513	0.20(0.07-0.62)	**0.005**	**0.00125**
rs2683511					
Genotypes					
CC	1		1		
CT	1.25(0.47-3.35)	0.648	1.61(0.48-5.38)	0.433	
TT	-		-		
Alleles					
C	1		1		

T	1.08(0.58-2.02)	0.791	1.09(0.40-3.00)	0.856	
rs36056065					
Genotypes					
GG	1		1		
G/del	0.82(0.20-3.23)	0.777	1.47(0.28-7.51)	0.642	
del/del	0.63(0.23-1.68)	0.358	0.60(0.18-1.98)	0.405	
Alleles					
G	1		1		
del	0.83(0.28-2.43)	0.733	1.15(0.58-2.26)	0.682	
rs3792269					
Genotypes					
AA	1		1		
AG	0.29(0.06-1.33)	0.113	0.15(0.02-0.85)	**0.033**	**0.00825**
GG	-		-		
Alleles					
A	1		1		
G	0.33(0.12-0.88)	**0.027**	0.87(0.24-3.14)	0.835	
rs5708135					
Genotypes					
CC	1		1		
TC	0.84(0.08-8.06)	0.885	1.37(0.11-15.08)	0.800	
TT	0.85(0.08-8.49)	0.890	1.83(0.14-22.87)	0.637	
Alleles					
T	1		1		
C	2.15(1.14-4.04)	**0.017**	0.33(0.10-1.20)	0.056	
rs622342					
Genotypes					
AA	1		1		
CA	1.83(0.58-5.71)	0.297	2.48(0.51-12.05)	0.258	
CC	4.65(1.08-19.86)	**0.038**	5.84(1.00-34.07)	0.049	
Alleles					

A	1		1		
C	0.83(0.22-3.08)	0.784	1.78(0.89-3.52)	0.098	
rs72552763					
Genotype					
GG	1		1		
G/del	0.28(0.03-2.31)	0.240	0.11(0.01-1.56)	1.105	
del/del	-		-		
Allele					
G	1		1		
del	1.47(0.76-2.84)	0.784	1.06(0.20-5.61)	0.943	
rs889299					
Genotypes					
AA	1		1		
GG	3.55(1.11-11.33)	0.032	7.91(1.67-37.27)	**0.009**	**0.00225**
GA	2.66(0.87-8.10)	0.084	5.27(1.19-23.19)	**0.028**	**0.007**
Alleles					
A	1		1		
G	1.47(0.76-2.84)	0.249	1.02(0.48-2.15)	0.946	0.105

CI=confidence interval

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Epistatic interaction patterns between SNPs and their association with response to metformin/SU combination therapy

Epistatic interactions between *CPA6, PRPF31, SLC22A1, NBEA and SCNN1B* were analysed using Multifactor dimensionality reduction (MDR). The combination of rs21621459 (*CPA6*) and rs889299 (*SCNN1B*) demonstrated a high CVC score (6/10), and it was significantly associated with metformin/SU combination therapy outcome (p=0.0022). The combination of rs2162145 (*CPA6*) and rs622342 (*SLC22A1*) and rs889299 (*SCNN1B*) showed a low CVC score (5/10) (Table 4, S1).

Table 4: Interaction models among the NBEA, SLC22A1 and SCNN1B polymorphisms in T2D patients

Interaction Models	Training Score	Testing score	CVC	P-value
CPA6 rs2162145	0.6174	0.5099	7/10	0.0107
CPA6 rs2162145 and SCNN1B rs889299	0.6749	0.4385	5/10	0.0004
CPA6 rs2162145 , *SLC22A1* rs622342 and *SCNN1B* rs889299	0.7357	0.4742	6/10	**0.0001**

CVC= cross-validation consistency

The genotype combinations GA (rs889299) and TT CPA6 rs2162145, GA (rs889299) and TC (rs2162145), TT (rs2162145) and GG (rs889299) were prominently detected among patients with uncontrolled T2DM (HbA1c >7%). The combination GA (rs889299) and TT rs2162145 was associated with uncontrolled T2DM (Figure 1).
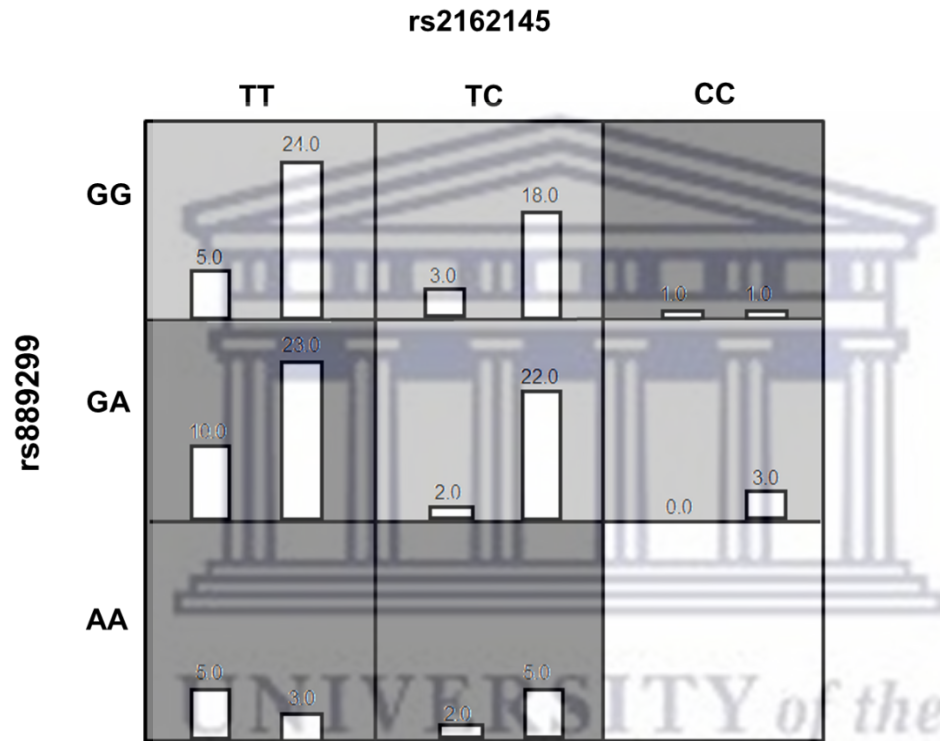
Figure 1: The best MDR model of interaction among rs2162145 and rs889299. The distributions of Controlled (left bars) and Uncontrolled (right bars) are illustrated for each combination of genotypes. Each cell represents genotype combinations. Dark grey cells represent genotype combinations implicated in uncontrolled T2DM in response to metformin/SU treatment. Light grey cell represent genotype combinations implicated in controlled T2D in response to metformin/SU combination therapy. White cells represent missing data.
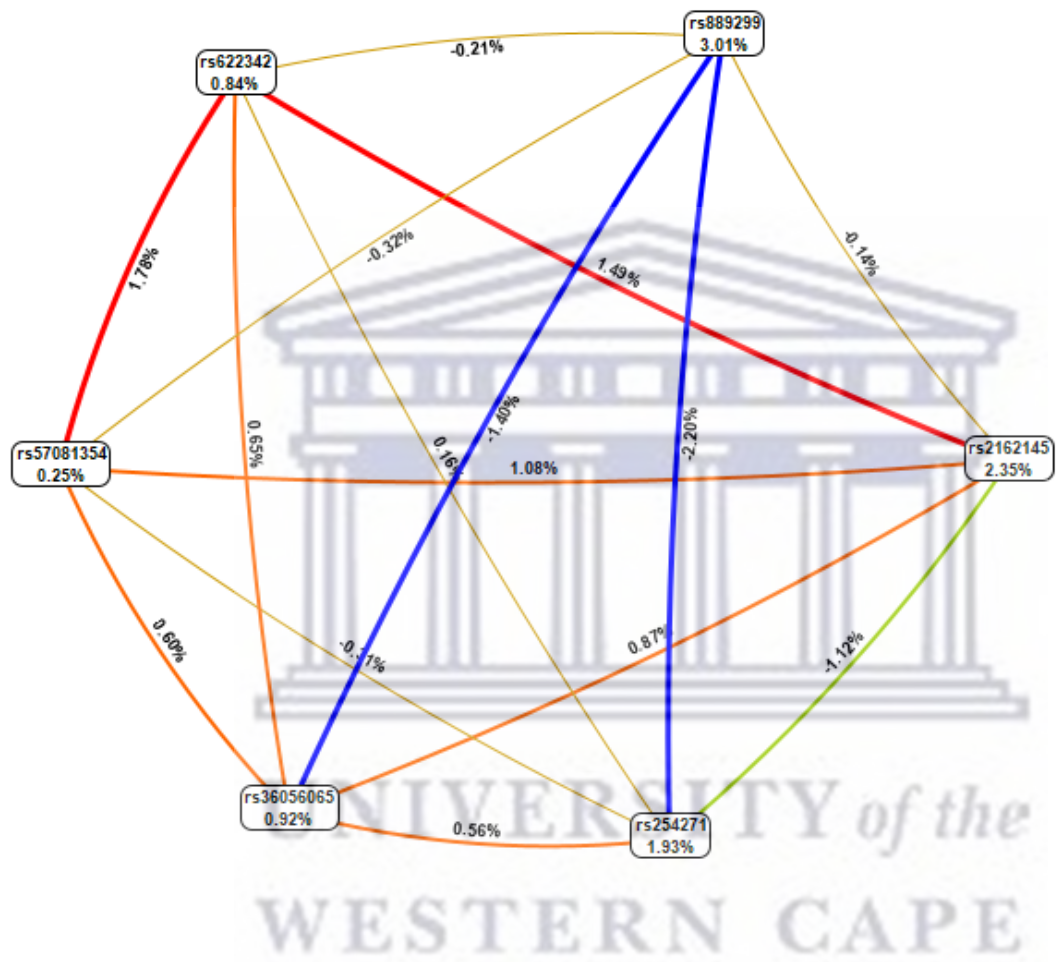
Figure 2: MDR combined attribute network showing all possible interactions between SNPs. Each color represents a possible interaction. Figures and line width indicate the strength of the interaction. Figures < 1 and thin lines represent weak interactions. The strongest interactions are represented by figures ≥1 and thick lines.

245

Discussion

The combination of metformin and SUs is among the most commonly prescribed dual therapies for the treatment of T2DM. Although widely prescribed, treatment outcome with oral anti-diabetic drugs differs strongly between individuals due to genetic factors. Accounting for these factors would lead to more personalised treatment regimens and help combat the increasing prevalence of uncontrolled T2DM. Therefore, the current study investigated the association of nine polymorphisms belonging to *SLC22A1, SP1, PRPF31, NBEA, SCNN1B, CPA6 and CAPN10* genes with glycaemic response to metformin/SU combination therapy. The study further assessed genetic interactions between these SNPs and glycaemic response to metformin/SU combination therapy among South African adults with T2DM.

In this study, we investigated the effect of two *SLC22A1* polymorphisms (rs36056065 and rs622342) on glycaemic response to metformin/SU combination therapy in patients with T2DM. The CC genotype of rs622342 was significantly associated with uncontrolled T2DM. The *SLC22A1* gene plays a crucial role in metformin transport. As such, polymorphisms in this gene have been associated with metformin response among patients with T2DM [35]. In a South India population, Umamaheswaran et al. [36] demonstrated that carriers of allele C of rs622342 showed decreased response to metformin therapy. It was further demonstrated that this effect was more pronounced among carriers of two copies of the C allele [36]. Furthermore, Naja et al. [37] showed that Lebanese carriers of the AC or the AA genotype exhibited better glycaemic control in individuals with T2DM undergoing metformin/SU combination therapy. Similar effects were observed among Egyptian patients of T2DM [37]. These findings warrant the use of this polymorphism as a predictor of metformin/SU efficacy among patients of African origin with T2DM.

We investigated the effect of rs2162145 (*CPA6*), rs57081354 (*NBEA*) and rs254271 (*PRPF31*) on glycaemic response to metformin/SU combination. Our findings suggest that Swati and Zulu carriers of the CC genotype of rs2162145 and C allele of rs57081354 were more likely to exhibit uncontrolled T2DM in response to metformin/SU combination therapy. Whereas carriers of the minor allele C of rs254271 were more likely to exhibit controlled T2DM in response to metformin/SU combination therapy. In a mixed cohort composed of patients of European and African descent (African American), Rotroff et al [25] showed that carriers of the CT and TT genotypes of rs2162145 may have a better response to metformin in comparison to carriers of the CC genotype. The study further demonstrated that carriers of the C allele of rs57081354 may have a decreased response to metformin [25]. With regards to rs254271, the authors demonstrated that Caucasian carriers of the CG and CC genotypes may have decreased response to metformin as compared to patients with genotype GG. Of note, this SNP was monomorphic among African Americans [25]. Literature suggest that African Americans are admixed in their African components of ancestry, with the majority contributions being from West and West-Central Africa. As such, the genetic architecture of African Americans is distinct from that of Africans [38]. Also, present day South Africans exhibit extensive genomic diversity in comparison to other populations groups [39]. On these grounds, it is possible for rs254271 to demonstrate different expression patterns among different groups of African origin. Genetic diversity may also be the reason for the disparities observed in the direction of association of the minor allele among people of European ancestry and South Africa Nguni people. Additional investigations conducted in a more diverse South African cohort are required to confirm the clinical impact of rs254271, rs2162145 and rs57081354 and further explore their potential as predictors of glycaemic response to anti-diabetic drugs.

In addition, the G allele and AG genotype of rs3792269 (*CAPN10*) were significantly associated with controlled T2DM in response to metformin/SU combination therapy in our

cohort of South African Nguni (Swati and Zulu). The CAPN10 gene encodes calcium-dependent intracellular protease that is important in calcium-regulated signaling pathways [21]. The variant rs3792269 of *CAPN10* was previously associated with the preventive effect of metformin on the development of T2DM in subjects with pre-diabetic dysglycaemia [40]. This effect was observed among Caucasian carriers G allele who reside in Slovakia. However, the preventative effect of this SNP on the development of T2DM is yet to be established among people of African origin. In addition to preventing T2DM, it was demonstrated that European carriers of the minor allele G had a smaller probability of achieving HbA1c <7 % and they had a smaller reduction in HbA1c during the first 6 months of metformin treatment [21]. The differences observed in both studies could be explained by several factors. For instance, our study sampled patients who were on combination therapy, while Tkáč et al [21] investigated patients on metformin monotherapy. Also, our study population of South African Nguni is different from the Central European Caucasian patients that were used in the reference study. While the direction of association of the genotypes and minor allele differed from previous findings, this SNP is proving to be of relevance in anti-diabetic treatment response among patients with T2DM.

In the present study, the GA and GG genotype of rs889299 were associated with uncontrolled T2DM in response to metformin/SU combination therapy. The A allele of the variant was previously associated with oedema in diabetic patients treated with Farglitazar and glibenclamide among Caucasians who reside in the United Kingdom (UK) [30]. The SNP rs889299 occur on the intronic region of *SCNN1B*, a gene that is responsible for providing instructions for the construction of the beta-subunit of ENac [30]. The activity of ENac is regulated by ATP-binding cassette protein such as the K channel-associated sulfonylurea receptor [31]. The K channel-associated sulfonylurea receptor is responsible for maintain energy balance within a living cell [14,41]. Sulfonylureas bind specific sites of this receptor,

248

thereby blocking the inflow of K^+ and stimulating the diffusion of Ca^+ into the cytosol. This activity leads to the contraction of the filaments of actomyosin responsible for the exocytosis of insulin granules, which is therefore promptly secreted in large amounts [14,41]. While metformin has no effect on *SCNN1B*, its mechanism of action complements that of SUs by improving insulin sensitivity [11]. On these grounds, rs889299 can be used as a predictor for SU monotherapy or metformin/SU combination therapy. There is currently no record of the effect of rs889299 on glycaemic response to metformin and SU monotherapy or combination therapy. To the best of our knowledge, this is the first study to explore the effect of this polymorphism on glycaemic response to metformin/SU combination therapy in a population of African origin.

In the current study, we investigated genetic interactions between rs5708135 (*NBEA*), rs2162145 (*CPA6*), rs36056065 (*SLC22A1*), rs622342 (*SLC22A1*) and rs889299 (*SCNN1B*) and their effect on metformin/SU combination response. An interaction between rs2162145 and rs889299 was observed. Furthermore, the combination of TT (rs2162145) and GG (rs889299) as well as GA (rs889299) and TT (rs2162145) were prominently detected among uncontrolled patients. The GA (rs889299) and TT (rs2162145) combination was implicated in uncontrolled T2DM. Of note, the TT genotype of rs2162145 was associated with better response to metformin. The effect of this SNP may depend on the presence of rs889299, suggesting that both SNPs may synergistically influence glycaemic response to metformin/SU combination therapy among South African Nguni patients. The importance of gene-gene or SNP-SNP interactions is gaining recognition in the field of pharmacogenomics [42]. Epistatic interactions between rs594709 and rs2289669 in metformin efficacy among Chinese patients with T2D were reported by Xiao et al (2016). Furthermore, Naja et al [29] reported interactions between rs622342 (*SLC22A1*) and *CYP2C9*2* and *CYP2C9*3* associated with reduced levels of HbA1c in response to metformin/SU combination therapy among Lebanese patients. Only a

few studies have explored this phenomenon with regards to anti-diabetic drugs, however; the importance of epistasis in anti-diabetic therapy is clearly identifiable. These findings have laid a foundation for the investigation of the complex interactions among genetic, and epigenetic factors that influence glycaemic response in metformin/ SU combination therapy among T2DM patients.

Limitations

Few limitations of the study cannot be ignored. The cross-sectional design does not allow for causal relationship to be established. Wide confidence interval and the high CVC score for the MDR model of interaction observed in the relationship between the SNPs and the glycaemic control is due largely to the small sample size. The absence of rs2683511 (TT), rs3792269 (GG) and rs72552763 (del/del), which were needed to better assess interaction effects that exist between these SNPs and glycaemic response to metformin/SU combination therapy. This is a health facility-based study with strict selection criteria (participants should have been initiated on combination therapy of metformin and SUs for at least a year at the time of the study). Thus, men were under-represented in the study due to their low utilisation of health facilities in the region. Low utilisation of health facilities by men has been reported extensively across South Africa. The proportion of men who utilise the healthcare system in the Eastern Cape Province ranged from 28.30% to 32.16 as demonstrated by Adeniyi et al [43] and Owolabi et al [44]. Motala et al [45], Adebolu et al [46] and Olowe et al [47] reported utilisation rates ranging between 20.48% and 30.00% in the KwaZulu Natal province. In the Western Cape, Erasmus et al. [48]and Peer et al [49] reported rates ranging from 19.36 to 35.66%. Future studies should specifically target men and other ethnic populations at the community level in order to gain better understanding of the associations between SNPs on glycaemic response to metformin/SU combination therapy. Notwithstanding of these limitations, this study provides new insights into pharmacogenomics of metformin/SUs in South African adults with T2DM.

In addition, this study has opened doors for pharmacogenomic studies in the ethnically-diverse population of South Africa.

Conclusion

This study reports the association of rs2162145 (CC), rs889299 (GA and GG) and SLC22A1 rs622342 (CC) and rs57081354 (C) with uncontrolled T2DM in response to metformin/SU combination therapy in South Africa. The study also reports an association of rs254271 (C), rs3792269 (G allele and genotype AG) with controlled T2DM. Furthermore, the study established an interaction between rs889299 and rs2162145 that is implicated in metformin/SU treatment outcome in an indigenous South African population. Further, pharmacogenomics and functional investigations should be conducted in a bigger South African cohort to confirm the effects of these genetic variants on metformin/SU combination therapy and provide more powerful evidence for their use as predictors of anti-diabetic treatment response.

Acknowledgements

Authors' Contributions

CM, BP, JJO and MB conceptualised, designed and implemented the study protocol. CM and OVA analysed the data and drafted the manuscript. All authors revised and approved the final draft of the manuscript for submission.

Conflict of interest

The authors declare no conflict of interest.

251

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Chapter 9 – Conclusion and Future Prospects

Hypertension and DM are rapidly emerging as public health problems in developing countries, and both are major risk factors for cardiovascular diseases. Furthermore, both diseases impose a major burden resulting in high mortality and morbidity, decreased life expectancy, reduced quality of life, as well as individual and national income losses. Both diseases are known to co-exist in patients. Literature suggests that there is a strong correlation between changing lifestyle factors and the risk of hypertension and DM. Moreover, challenges in the adequate treatment and management of both diseases are dependent on factors at the patient, clinician and healthcare system levels. Epidemiologic studies have important clinical impact and have led to an increasing appreciation of scientific basis for clinical and public health practice.

Epidemiological data is also important in informing Precision Medicine, an innovative approach that takes into account individual differences in patients' genes as well as environments and lifestyles. With the current paradigm shift in patient care towards precision medicine, HPT and DM management could be improved by stratifying responders and non-responders to treatment, in terms of drug choice and dose, based on patients' genetic background. Although, Africa is at the forefront of the DM and hypertension epidemic, epidemiological data as well as genomic data is lacking. This has led to major setbacks in the understanding of the epidemiological and genomic factors driving the prevalence of uncontrolled hypertension and DM in the region. The present research project examined socio-demographic, clinical and genetic determinants of uncontrolled hypertension and uncontrolled DM among indigenous South African adult patients living in rural areas of the Mpumalanga and Eastern Cape Province. The main socio-demographic and clinical factors, which showed association with failing to achieve blood pressure and glycaemic targets, included unfavorable lifestyle and/or diets, as well as dyslipidemia. Measures aimed at changing dietary and lifestyle

patterns should, therefore, be implemented even when undergoing treatment taking drug treatment for HPT Hypertension and DM.

We found a high prevalence of uncontrolled hypertension among our study population, which may be attributed to low HDL-C, inadequate physical activity, and the presence of obesity. The determinants that we have presented need to be addressed through collaborative efforts between patients, clinicians as well as decision makers in the healthcare system, in order to improve the overall outcomes of clinical care among hypertensive patients in the rural areas of the Mpumalanga province. To the best of our knowledge, this is the first study to assess and report the prevalence of uncontrolled hypertension and its determinants in the Mkhondo Municipality.

Using a candidate gene approach, we investigated the effect of SNPs on blood pressure response to hydrochlorothiazide treatment among patients belonging to the indigenous Nguni tribes. We detected seventeen SNPs in hydrochlorothiazide associated genes among the Xhosa population, and only two SNPs among Zulu and Swati participants. The variant alleles of WNK1 rs2107614 and rs2776546 were independently associated with uncontrolled hypertension among Xhosa participants. Among Zulu and Swati participants, the variant allele T of YEATS4 rs7297610 was independently associated with uncontrolled hypertension. It is important to note that this is the first South African study to establish an association between the YEATS4 rs7297610 and blood pressure response to hydrochlorothiazide treatment. In addition to hydrochlorothiazide SNPs, this study also reported the detection of five SNPs (rs2239050, rs2246709, rs4291, rs1042713 and rs10494366) in amlodipine associated genes among the indigenous Xhosa speaking tribe of South Africa. None of these SNPs were detected among Zulu and Swati participants. Furthermore, we established an association between the TA genotype of rs4291 and blood pressure response to amlodipine treatment among the Xhosa cohort. The research project also included the investigation of the effect of SNPs in enalapril

257

associated genes on blood pressure response to enalapril treatment. We reported five SNPs that were exclusively expressed among the Zulu and Swati cohort. Furthermore, an association of genotypes rs1042714 (GC) and rs1799722 (CT) with blood pressure response to enalapril treatment was established. In the same cohort, the C allele of rs699947 was associated with uncontrolled hypertension in response to enalapril treatment. For the first time in an indigenous South African cohort, an interaction between ABO rs495828, NOS3 rs2070744 and VEGFA rs699947 associated with enalapril treatment outcome was established using MDR. These findings suggest that these SNPs may synergistically influence blood pressure response to enalapril treatment. Overall, the findings of this part of the research project highlight the relevance of comprehensively characterising highly diverse populations, particularly those of African origin in order to facilitate promote pharmacogenomics studies in the African continent. These findings have also provided substantial evidence to suggest the use of polymorphisms within in genes directly or indirectly implicated in ACE inhibitor, diuretic and CCBs pathways as predictors of blood pressure response among African patients. However, the mechanism in which these SNPs influence blood pressure in response to the different classes of anti-hypertensive drugs is unknown. Further elucidation of the exact mechanism in which these SNPs affect blood pressure in response to the number of ant-hypertensive drugs under the different classes could ultimately aid in advancing our understanding of the extent in which SNPs influence blood pressure response, and improve the identification of new drug targets. In addition, our findings might open doors for more pharmacogenomics studies, which could lead to improved and personalized anti-hypertensive treatment in the ethnically diverse population of South Africa.

In addition to epidemiological and genetic factors associated with uncontrolled hypertension, we investigated epidemiological and genetic determinants of uncontrolled DM. We found a high prevalence of poor glycaemic control among participants who were residents of the

Mkhondo Municipality that may be attributed to religious affiliation, fast food consumption and dyslipidemia. Furthermore, about half of the study participants exhibited very poor glycaemic control (HbA1c ≥ 9%), which was predominantly observed among younger adults. Using the same study cohort, we further analysed polymorphisms that were associated with metformin and SU combination therapy. Nine SNPs, including rs2162145, rs2282143, rs254271, rs2683511, rs3792269, rs57081354, rs72552763, rs36056065 and rs622342 were detected among participants. The genotypes of CPA6 rs2162145 (CC), SCNN1B rs889299 (GA and GG) and SLC22A1 rs622342 (CC) and variant allele of NBEA rs57081354 (C) were associated with uncontrolled T2DM in response to metformin/SU combination therapy. We also reported an association of PRPF31 rs254271 (C), CAPN10 rs3792269 (G allele and genotype AG) with improved T2DM control. In addition, the study established an interaction between SCNN1B rs889299 and CPA6 rs2162145 that is implicated in metformin/SU treatment outcome in an indigenous South African population. However, the limitations of the study cannot be ignored. The cross-sectional design does not allow for causation to be established. Also, men are under-represented in the study due to their low utilisation of health facilities in the region. The absence of rs2683511 (TT), rs3792269 (GG) and rs72552763 (del/del), which were needed to better assess interaction effects that exist between these SNPs and glycaemic response to metformin/SU combination therapy. Our sample size was relatively small, thus future pharmacogenomics and functional investigations should be conducted in a bigger South African cohort to confirm the effects of these genetic variants on the efficacy of metformin/SU combination therapy, and provide more evidence for their use as predictors of anti-diabetic treatment response. Notwithstanding of these limitations, this study has opened doors for pharmacogenomics studies in the ethnically-diverse population of South Africa. Moreover, these findings could be used to guide local authorities and clinicians in crafting and

implementing appropriate interventions to improve clinical outcomes in people with DM in the region.

Like many developing countries, South Africa stands to benefit from pharmacogenomic and precision Medicine research. As a result, pharmacogenomics was specifically highlighted in The National Biotechnology Strategy for South Africa and it is also advocated by the South African Department of Science and Technology (DST). The current genomic challenge is to understand genotype-environment and genotype-drug interactions in our genetically diverse South African population, and to translate this knowledge into clinical applications that can be applied in the management of both DM and hypertension. The data presented thus far has highlighted the unique genetic profiles in the Nguni speaking tribe, that is displayed by allele frequencies that differ from each ethnic group as well as other African tribes. This also emphasises the need for appropriate genotyping platforms that will capture the genetic diversity presented by each population. Future Pharmacogenomics and Precision Medicine studies should adopt a GWAS approach and focus on early detection, prevention and adequate treatment of both diseases. In order to achieve this goal, future research needs to be carefully planned in order to enable the efficient use of limited funding. Also, researchers should adopt a holistic view on personalised medicine that goes beyond a genetic approach and include all aspects of treatment response including proteomics, epigenetics and epidemiology. Should South Africa succeed in this aspect, the knowledge generated will be used as a foundation for African-specific pharmacogenomics studies.

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Appendix 1-Ethical Clearance

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06 March 2019

Prof M Benjeddou
Biotechnology
Faculty of Natural Sciences

Ethics Reference Number: BM16/5/19

Project Title: Precision Medicine: Pharmacogenomics and development of individualized drug therapy for sub-Saharan African populations.

Approval Period: 15 February 2019 – 15 February 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
University of the Western Cape

BMREC REGISTRATION NUMBER -130416-050

FROM HOPE TO ACTION THROUGH KNOWLEDGE

Appendix 2-Approval from the Mpumalanga Department of Health

Appendix 3-Submission Evidence

A manuscript number has been assigned to Single Nucleotides Polymorphisms in Amlodipine associated genes and their associations with blood pressure control among South African adults with Hypertension

Inbox ×

Pharmacogenetics and Genomics <em@editorialmanager.com>   Thu, Dec 17, 2020, 12:10 PM

to me

Dear Miss Masilela,

Your submission entitled "Single Nucleotides Polymorphisms in Amlodipine associated genes and their associations with blood pressure control among South African adults with Hypertension" has been assigned the following manuscript number: PGEN-2020-112.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author.

https://www.editorialmanager.com/pgen/

username: CMMASILELA
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Thank you for submitting your work to Pharmacogenetics and Genomics.

Kind Regards,

Prachi Deshpande
Editorial Coordinator
Pharmacogenetics and Genomics

Appendix 4-Submission Evidence

⊞ Action ⚠	Manuscript Number ▲▼	Title ▲▼	Initial Date Submitted ▲▼	Status Date ▲▼	Current Status ▲▼
Action Links	MD-D-20-10812	Association of Five Single Nucleotide Polymorphisms with Enalapril Treatment Response among South African Adults with Hypertension	Nov 2 2020 1:39PM	Jan 15 2021 9:48AM	Under Review

http://etd.uwc.ac.za/

Appendix 5-Reviwers comments and Response from Authors

Reviewer #2: I would like to thank the authors for their tremendous efforts in providing this work, which predicts the response to enalapril treatment in patients with hypertension based on 5 SNPs. However, I have some questions and requests that i would like to check prior to accepting this manuscript.

1- There are many factors that could result in a change in the response to antihypertensive medications, including obesity, alcohol consumption, sedentary lifestyle, and the use of multiple antihypertensive medications. Have you collected this information (besides the multiple hypertensive medications) at baseline? If yes, please provide them in Table 1.

Answer: The suggested variables have been added to table 1.

2- In the regression model, you reported the adjusted odds ratio, without describing in the text which confounding variables you controlled for? What were the variables that you have controlled in the multiple regression model?

Answer: The variables that were used to adjust the regression model are listed in the in the methods section.

3- This could be quite a challenge, given the fact that the majority of your population used enalapril with three other antihypertensive meds (66.90%). How certain can you be that the SNPs would predict the response to enalapril where only 8.8% of patients were given enalapril with 1 antihypertensive med?

Answer: Thank you for the comment. The authors recognize this as a limiting factor. However, obtaining samples from patients who are on enalapril monotherapy is not feasible in the study setting. The South African guidelines for hypertension treatment recommend thiazide diuretics as initial therapy and enalapril is introduced at a later stage as a second- or third-line drug

(Seedat et al., 2014)[36]. As a result, interference from other anti-hypertensive drugs could not be avoided. Nevertheless, this study has laid a foundation for African-specific genomic studies and it has the potential to guide hypertension treatment in the future.

36. Seedat YK, Rayner BL, Veriava Y. South African hypertension practice guideline 2014. Cardiovascular journal of Africa. 2014 Nov;25(6):288.

4- Please provide a table of enalapril-associated SNPs based on the following variables: (controlled vs uncontrolled hypertension) and (enalapril+1 vs. enalapril+2 vs. enalapril+3 drugs).

Answer: Thank you for the suggestion. The suggested table has been provided (Table 3).

Appendix 6-Open Access Policy

267

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