Cross-sectional study of the association of 5 single nucleotide polymorphisms with enalapril treatment response among South African adults with hypertension

Charity Masilela, PhD[a,∗,‡], Brendon Pearce, PhD[a], Joven Jebio Ongole, MBCHB, MSC, MMED, FCFP[b], Oladele Vincent Adeniyi, FCFP, MMED, MPHIL, PhD[c], Rabia Johnson, PhD[d,e], Mongi Benjeddou, PhD[a]

Abstract
This study investigates the association of 5 single nucleotide polymorphisms (SNPs) in selected genes (ABO, VEGFA, BDKRB2, NOS3, and ADRB2) with blood pressure (BP) 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.

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 BP ≥140/90 mm Hg. The association between genotypes, alleles, and BP response to treatment was determined by fitting multivariate logistic regression model analysis, and genetic interactions between SNPs were assessed by multifactor dimensionality reduction.

Majority of the study participants were female (75.00%), Xhosa (78.87%), and had uncontrolled hypertension (69.37%). All 5 SNPs were exclusively detected among Swati and Zulu participants. In the multivariate (adjusted) logistic model analysis, ADRB2 rs1042714 GC (adjusted odds ratio [AOR] = 2.31; 95% confidence interval [CI] 1.02–5.23; P = .044) and BDKRB2 rs1799722 CT (AOR = 2.74; 95% CI 1.19–6.28; P = .017) were independently associated with controlled hypertension in response to enalapril. While the C allele of VEGFA rs699947 (AOR = 0.37; 95% CI 0.15–0.94; P = .037) was significantly associated with uncontrolled hypertension. A significant interaction between rs699947, rs495828, and rs2070744 (cross-validation consistency = 10/10; P = .005) in response to enalapril was observed.

We confirmed the association of rs1042714 (ADRB2) and rs1799722 (BDKRB2) with controlled 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.

Abbreviations: ABO = histo-blood group ABO, ACE = angiotensin-converting enzyme, ADRB2 = beta-2 adrenergic receptor, AOR = adjusted odds ratio, BDKRB2 = Bradykinin receptor B2, BP = blood pressure, CI = confidence interval, MDR = multifactor dimensionality reduction.
1. Introduction

The prevalence of hypertension has increased rapidly in the past decade, reaching epidemic proportions in the lower and middle-income countries. This increasing trend has also been reported in South Africa, especially among the predominant black ethnic group. The age-standardized prevalence of hypertension was estimated at 39.9% among urban dwelling Black South Africans. Furthermore, the high burden of hypertension is attributed to the epidemiologic and nutritional transitions characterized by urbanization 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. 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.

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 (BP) response to anti-hypertensive treatment. However, there is currently insufficient evidence on the specific SNPs that predict BP 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. It exerts its BP lowering properties by suppressing the formation of angiotensin II thereby reducing arterial pressure, preload, and afterload on the heart. 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. 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. 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’-monophosphate. The latter triggers the NO system to activate vasodilatation by increasing arginine uptake.

Polymorphisms in genes coding proteins that are implicated in ACE inhibitor induced vasodilation have been associated with variable BP in response to enalapril. For instance, the rs1799722 polymorphism sits on the promoter region of Bradykinin receptor B2 (BDKRB2) that encodes the BDKRB2 protein. The rs699947 polymorphism is found on the VEGFA gene, which encodes the VEGF protein. On the other hand, rs1042714 and rs2070744 are found on the ADRB2 gene and NOS3 gene, respectively. The NOS3 gene encodes the enzyme NOS3, while ADRB2 encodes the cell membrane-spanning ADRB2 protein. 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. Moreover, GG+CG of rs1042714 (ADRB2) were associated with increased enalapril sensitivity among Europeans with left ventricular hypertrophy. Whereas carriers of the TT genotype of rs2070744 (NOS3) showed reduced response to enalapril treatment. 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. 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 (ABO) system transferase is an enzyme with glycosyltransferase activity, encoded by the ABO gene. The gene was previously associated with variation in plasma ACE activity, inflammation, increased risk of hypertension, and ACE inhibitor-induced cough. 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 advance 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.

2. Materials and methods

2.1. 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.

2.2. 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.

2.3. 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) and height to the nearest of 0.1 cm using a mounted stadiometer, with participants wearing minimal clothing. The BP of each participant was measured 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 categorize participants into 2 groups: controlled (BP < 140/90 mmHg) and uncontrolled (BP ≥ 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.

2.4. 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 sodium chloride 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 2mL 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).

2.5. Selection of SNPs and genotyping

Five SNP previously associated with enalapril efficacy in individuals with hypertension were selected using Pharmacogenomics Knowledge Base.28 Ensembl29 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 Polymerase chain 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 mass spectrometry was used to identify the SNP of interest.

2.6. Statistical analysis

Statistical analyses were performed using IBM Statistical Package for Social Science (SPSS) Version 25 for Windows (IBM Corps, Armonk, NY). The general characteristics of the participants were expressed as frequency count (percentages). The associations between alleles, genotypes, and BP 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. Results for the unadjusted logistic regression model analysis were expressed as crude odds ratios and adjusted odds ratios (AORs) for the adjusted logistic regression model analysis. Odds ratios >1 indicated an association with controlled hypertension, while odds ratios <1 indicated an association with uncontrolled hypertension. A P value less than .05 was considered statistically significant.

Minor allele frequency and Hardy–Weinberg equilibrium 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 multifactor dimensionality reduction (MDR) version 3.0.2. Only 2 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 score and P values. P values were calculated using x² test, values < .05 were deemed significant.

3. Results

3.1. 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 BP (≥140/90 mmHg) (Table 1).

3.2. Expression patterns of SNPs across 3 population groups

The expression frequency of 5 SNPs was evaluated in 3 populations (Swati, Xhosa, and Zulu). All 5 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 x²-test.

All 5 SNPs were predominantly detected among participants who exhibited uncontrolled hypertension. However, a significant relationship between BP response and rs495828 (P = .033) as well as rs699947 (P = .004) was established. On the other hand, rs1042714 and rs1799722 were detected among 48.78% and
53.38% of participants who were prescribed enalapril + 3 drugs, respectively. The 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.

### 3.3. Association of enalapril-associated SNPs with controlled hypertension

In the multivariate logistic regression model (unadjusted) analysis, the GC genotype of rs1042714 was associated with controlled hypertension in response to enalapril treatment [crude odds ratio = 2.41 (1.17–4.94); \( P = .016 \)]. 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); \( P = .044 \)] and rs1799722 CT [AOR = 2.74 (1.19–6.28); \( P = .017 \)] were independently associated with controlled hypertension in response to enalapril. Furthermore, the C allele of rs699947 [AOR = 0.37 (0.15–0.94); \( P = .037 \)] was significantly associated with uncontrolled hypertension in response to enalapril (Table 4).

### 3.4. Interaction between enalapril-associated SNPs

Interactions among the ADRB2, ABO, NOS3, BDKRB2, VEGFA polymorphisms were assessed using MDR. The combination of rs699947 (VEGFA), rs495828 (ABO), and rs2070744 (NOS3) demonstrated a high cross-validation consistency score (10/10) and was significantly (\( P = .0005 \)) associated with BP response to enalapril (Table 5).

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 (Fig. 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 (yrs)                          |                     |                |
| 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 mm Hg                      | 197                 | 69.37          |
| ≥140/90 mm Hg                      | 87                  | 30.63          |
| Number of anti-hypertensive drugs  |                     |                |
| Enalapril + 1                      | 25                  | 8.80           |
| Enalapril + 2                      | 69                  | 24.29          |
| Enalapril + 3                      | 190                 | 66.90          |

**BMI** = body mass index.

### Table 2

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

| DbSNP Gene | Zulu (n; %) | Swati (n; %) | Xhosa (n; %) | Male (n; %) | Female (n; %) | <55 yrs | 55–65 yrs | >65 yrs |
|------------|-------------|--------------|--------------|-------------|---------------|---------|-----------|---------|
| rs1042714  | ADRB2       | 91 (75.83)   | 32 (78.05)   | 0 (0.00)    | 21 (29.58)    | 33 (47.77) | 37 (38.95) | 53 (48.18) |
| No         | 29 (24.17)  | 09 (21.95)   | 123 (100)    | 50 (70.42)  | 46 (58.23)    | 58 (61.05) | 57 (51.82) |
| rs1799722  | BDKRB2      | 112 (93.33)  | 39 (95.12)   | 0 (0.00)    | 25 (35.21)    | 46 (58.23) | 41 (43.16) | 64 (58.18) |
| No         | 08 (6.67)   | 02 (4.88)    | 123 (100)    | 46 (64.79)  | 33 (41.77)    | 54 (56.84) | 46 (41.82) |
| rs2070744  | NOS3        | 81 (67.50)   | 33 (80.49)   | 0 (0.00)    | 18 (25.35)    | 29 (36.71) | 33 (34.74) | 52 (47.27) |
| No         | 39 (32.50)  | 08 (19.51)   | 123 (100)    | 53 (74.65)  | 50 (63.29)    | 62 (65.26) | 58 (52.73) |
| rs495828   | ABO         | 114 (95.00)  | 39 (95.12)   | 0 (0.00)    | 24 (33.81)    | 46 (58.23) | 42 (44.21) | 65 (59.09) |
| No         | 06 (5.00)   | 02 (4.87)    | 123 (100)    | 47 (66.19)  | 33 (41.11)    | 53 (55.79) | 46 (40.91) |
| rs699947   | VEGFA       | 87 (72.5)    | 31 (75.60)   | 0 (0.00)    | 20 (28.17)    | 33 (41.11) | 33 (34.74) | 52 (47.27) |
| Yes        | 33 (27.5)   | 10 (24.39)   | 123 (100)    | 51 (71.83)  | 46 (58.23)    | 62 (65.26) | 58 (52.73) |

ABO = histo-blood group ABO, ADRB2 = beta-2 adrenergic receptor, BDKRB2 = Bradykinin receptor B2, NOS3 = nitric oxide synthase, SNP = single nucleotide polymorphism, VEGFA = vascular endothelial growth factor A.
4. Discussion
There is substantial variation in BP 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 5 SNPs with BP 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 BP response to enalapril among South African adults with hypertension.

South African Nguni people are classified into 4 sub-groups, Swati, Ndebele, Xhosa, and Zulu. Together, they represent two-thirds of South Africa’s Black population.\(^31\) The 4 groups are

| 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 Controlled (n; %) | BP Uncontrolled (n; %) | \(P\) value | Enalapril + 1 (n; %) | Enalapril + 2 (n; %) | Enalapril + 3 (n; %) | \(P\) value |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| rs1042714       | 51 (41.46)       | 72 (58.54)       | .275            | 17 (13.82)       | 46 (37.40)       | 60 (48.78)       | .174            |
| rs1799722       | 59 (39.07)       | 92 (60.93)       | .954            | 22 (14.57)       | 50 (33.11)       | 79 (52.32)       | .743            |
| rs2070744       | 46 (40.35)       | 68 (59.65)       | .621            | 15 (13.16)       | 43 (37.72)       | 56 (49.12)       | .195            |
| rs495828        | 57 (37.25)       | 96 (62.75)       | .033            | 21 (13.72)       | 53 (34.65)       | 79 (51.63)       | .135            |
| rs699947        | 54 (45.76)       | 64 (54.24)       | .004            | 16 (13.56)       | 42 (35.59)       | 60 (50.85)       | .567            |

BP = blood pressure, SNP = single nucleotide polymorphism.

| Table 4 | Independent association of SNPs associated with enalapril and controlled hypertension (multivariate logistic regression model). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| DbSNP | Unadjusted odds ratios (95% CI) | \(P\) value | Adjusted odds ratios (95% CI) | \(P\) value |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| All | rs1042714 | GG 1 | – | – | – | – |
| Genotypes | CC 1 | 2.41 (1.17–4.94) | 0.016 | 2.31 (1.02–5.23) | .044 |
| Alleles | G 1 | 0.84 (0.37–1.72) | 0.574 | 0.94 (0.40–2.26) | .903 |
| rs1799722 | TT 1 | 0.85 (0.35–2.02) | 0.729 | 0.97 (0.37–2.56) | .960 |
| Genotypes | CT 1 | 1.90 (0.94–4.02) | 0.071 | 2.74 (1.19–6.28) | .017 |
| Alleles | C 1 | 1.53 (0.93–2.53) | 0.093 | 1.31 (0.72–2.38) | .368 |
| rs2070744 | TT 1 | 0.60 (0.25–1.47) | 0.272 | 0.73 (0.25–2.16) | .582 |
| Genotypes | TC 1 | 0.61 (0.22–1.68) | 0.346 | 0.76 (0.22–2.56) | .667 |
| Alleles | C 1 | 0.73 (0.33–1.61) | 0.442 | 0.69 (0.30–1.58) | .386 |
| rs495828 | GG 1 | – | – | – | – |
| Genotypes | TT 1 | 2.84 (0.58–13.91) | 0.197 | 2.10 (0.36–12.10) | .403 |
| Alleles | G 1 | 1.40 (0.50–3.19) | 0.512 | 1.26 (0.42–3.77) | .479 |
| rs699947 | TT 1 | 1.15 (0.49–2.72) | 0.734 | 0.86 (0.32–2.29) | .772 |
| Genotypes | AA 1 | 0.93 (0.33–2.59) | 0.895 | 1.06 (0.31–3.56) | .923 |
| Alleles | A 1 | 1.21 (0.36–4.01) | 0.750 | 1.79 (0.44–7.18) | .407 |
| CA 1 | 0.43 (0.17–1.03) | 0.881 | 0.37 (0.15–0.94) | .037 |

CI = confidence interval, SNP = single nucleotide polymorphism.
to its role in vasodilatation.\cite{19,21} The current study investigated ADRB2 gene has been widely researched as a candidate gene for essential hypertension and anti-hypertensive drug response due 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 5 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.

ADRB2 are up-regulated in hypertension and largely polymorphic within the human population.\cite{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.\cite{19,21} The current study investigated the association of rs1042714 (ADRB2) with BP response 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.\cite{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.\cite{19} Ramipril falls under ACE inhibitors along with enalapril and they are assumed to influence BP in a similar pattern.\cite{13} 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.

VEGF is an important angiogenic factor encoded by the VEGFA gene.\cite{34} Furthermore, VEGF stimulates endogenous NO resulting in vasodilation.\cite{14} Owing to its role in BP 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 20mg/day enalapril.\cite{14} Haplotype analysis of rs699947, rs1570360, and rs2010963 demonstrated that carriers of the AGG haplotype had a better BP response following enalapril treatment. Whereas carriers of the CGG haplotype showed an opposite effect.\cite{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.

Bradykinin is a potent vasodilator that plays an important role in the pathophysiology of hypertension.\cite{15} Also, polymorphisms in the gene that mediated the production of bradykinin are implicated in enalapril treatment outcome.\cite{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.\cite{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.\cite{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 BP response in a large African cohort need to be conducted, in order to assess its suitability as a marker for enalapril treatment response.

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

| Interaction models | Training score | Testing score | CVC | P value |
|--------------------|----------------|---------------|-----|---------|
| VEGFA_r699947      | 0.5826         | 0.5826        | 10/10 | .3333   |
| NOS3_rs2070744, VEGFA_r699947 | 0.6356 | 0.5711 | 09/10 | .6251 |
| NOS3_rs2070744, ABO_rs495828, VEGFA_r699947 | 0.6776 | 0.5518 | 10/10 | .0005 |

ABO = histo-blood group ABO, CVC = cross-validation consistency, NOS3 = nitric oxide synthase, VEGFA = vascular endothelial growth factor A.
Epistatic interactions are likely to play a pivotal role in the pathophysiology of hypertension as well as therapeutic interventions. Epistatic interactions between rs2070744 (NOS3) and rs1799722 (BDKRB2) associated with BP response to enalapril were reported by Silva et al. Also, Oliveira-Paula et al. 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. Furthermore, the genotype combination of CC (rs699947), TT (rs2070744), and G/G (rs495828) were expressed more frequently among participants with controlled hypertension, while the combination of G/G (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. 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. 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 further 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.

4.1. 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, 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 2 genotypes, as a result it was excluded from the MDR interaction model. There are emerging evidence showing that variations in the BP over time have detrimental effects and are associated with increased risk of target organ damage, including brain, kidney, and heart. Future studies in this population should explore the associations between genetic factors and BP responses to enalapril.

5. Conclusion

The effect of an individual’s genetic variation on drug response is a major focus area of individualized medicine. In this study, we presented an association of 3 polymorphisms with variable enalapril response among patients with hypertension. These findings have provided substantial evidence for the use of polymorphism in genes directly implicated in ACE inhibitor pathways as predictors for enalapril response. Furthermore, this study has laid a foundation that will lead to the better understanding of gene–gene interactions within the ACE inhibitor pathway. In addition to a better understanding of the ACE inhibitor pathway, these findings will motivate a more personalized approach in the treatment of hypertension that is based on genetic testing. Thus, allowing medical practitioners to customize treatments based on an individual’s genetic makeup.

Acknowledgments

The authors would like to thank the study participants, Piet Retief Hospital, Thandukukhaya Community Health Center, Mkhondo Town Clinic, and the Department of Health of Mpumalanga.

Author contributions

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

Conceptualization: Charity Masilela, Brendon Pearce, Mongi Benjeddou.

Data curation: Oladele Vincent Adeniyi.

Formal analysis: Charity Masilela.

Funding acquisition: Mongi Benjeddou.

Investigation: Charity Masilela, Joven Jebio Ongole.

Methodology: Rabia Johnson.

Project administration: Charity Masilela and Brendon Pearce.

Supervision: Mongi Benjeddou.

Validation: Brendon Pearce, Oladele Vincent Adeniyi.

Writing – original draft: Charity Masilela.

Writing – review & editing: Oladele Vincent Adeniyi.

References

[1] Mills KT, Bundy JD, Kelly TN, et al. Global disparities of hypertension prevalence and control: a systematic analysis of population-based studies from 90 countries. Circulation 2016;134:441–50.

[2] Dewhurst MJ, Walker RW. Hypertension in Sub-Saharan Africa: prevalence, prescriptions, pitfalls and paradigms. J Hum Hypertens 2016;30:221–2.

[3] Rayner B. Hypertension: detection and management in South Africa. Nephron Clin Pract 2010;116:e269–73.

[4] Peer N, Steyn K, Lombard C, Gwebushe N, Levitt N. A high burden of hypertension in the urban Black population of Cape Town: the Cardiovascular Risk in Black South Africans (CRIBSA) study. PLoS One 2013;8:e78567.

[5] Yako YY, Balti EV, Matsha TE, et al. Genetic factors contributing to hypertension in African-based populations: a systematic review and meta-analysis. J Clin Hypertens (Greenwich) 2018;20:485–95.

[6] Luizon MR, Pereira DA, Sandrim VC. Pharmacogenomics of hypertension and pre-eclampsia: focus on gene–gene interactions. Front Pharmacol 2018;9:168.

[7] Oliveira-Paula GH, Pereira SC, Tanus-Santos JE, Lacchini R. Pharmacogenomics and hypertension: current insights. Pharmacogenomics Pers Med 2019;12:341.

[8] Alwi ZB. The use of SNPs in pharmacogenomics studies. Malays J Med Sci 2005;12:4–12.

[9] Chung C-M, Wang R-Y, Chen J-W, et al. A genome-wide association study identifies new loci for ACE activity: potential implications for response to ACE inhibitor. Pharmacogenomics J 2010;10:537–44.

[10] Luo J-Q, He F-Z, Wang Z-M, et al. SLCO1B1 variants and angiotensin converting enzyme inhibitor (Enalapril)-induced cough: a pharmacogenetic study. Sci Rep 2015;5:17233.
Masilela et al. Medicine (2021) 100:46

[11] Gomez HJ, Cirillo VJ, Irvin JD. Enalapril: a review of human pharmacology. Drugs 1985;30:13–24.

[12] Herrick AL, Waller PC, Berkini KE, et al. Comparison of enalapril and atenolol in mild to moderate hypertension. Am J Med 1989;86:421–6.

[13] Messerli FH, Bangalore S, Bavishi C, Rimoldi SF. Angiotensin-converting enzyme inhibitors in hypertension: to use or not to use? J Am Coll Cardiol 2018;71:1474–82.

[14] Oliveira-Paula GH, Lacchini R, Fontana V, Silva PS, Biagi C, Tanus-Santos JE. Polymorphisms in VEGFA gene affect the antihypertensive responses to enalapril. Eur J Clin Pharmacol 2015;71:949–57.

[15] Burrell LM, Phillips PA, Johnston CI. Mode of action of angiotensin converting enzyme inhibitors. Principles of Medical Biology (Vol. 8, 547-560) 1997; Elsevier.

[16] Silva PS, Fontana V, Luizon MR, et al. eNOS and BDKRB2 genotypes affect the antihypertensive responses to enalapril. Eur J Clin Pharmacol 2013;69:167–77.

[17] Flacco N, Segura V, Perez-Aso M, et al. Different β-adrenoceptor subtypes coupling to cAMP or NO/cGMP pathways: implications in the relaxant response of rat conductance and resistance vessels. Br J Pharmacol 2013;169:413–25.

[18] Gaio V, Nunes B, Fernandes A, et al. Genetic variation at the CYP2C19 gene associated with metabolic syndrome susceptibility in a South Portuguese population: results from the pilot study of the European Health Examination Survey in Portugal. Diabetol Metab Syndr 2012;4:25.

[19] Anthony EG, Richard E, Lipkowitz MS, Bhattacharaj V. Association of the ADRB2 (rs2053044) polymorphism and angiotensin-converting enzyme-inhibitor blood pressure response in the African American Study of Kidney Disease and Hypertension. Pharmacogenet Genomics 2015;25:444–9.

[20] Oliveira-Paula GH, Luizon MR, Lacchini R, et al. Gene-gene interactions among PRKCA, NOS3 and BDKRB2 polymorphisms affect the antihypertensive effects of enalapril. Basic Clin Pharmacol Toxicol 2017;120:284–91.

[21] Iaccarino G, Izzo R, Tramarchi V, et al. Beta2-adrenergic receptor polymorphisms and treatment-induced regression of left ventricular hypertrophy in hypertension. Clin Pharmacol Ther 2006;80:633–45.

[22] Yamamoto M, Tarasco MC, Cai E, Kobayashi H, Yamamoto F. ABO blood group A transferase and its codon 69 substitution enzymes synthesize FORS1 antigen of FORS blood group system. Scientific reports 2019;9:1–1.

[23] Luo J-Q, He F-Z, Luo Z-Y, et al. Rs495828 polymorphism of the ABO gene is a predictor of enalapril-induced cough in Chinese patients with essential hypertension. Pharmacogenet Genomics 2014;24:306–13.

[24] Gassó P, Mas S, Álvarez S, et al. A common variant of the ABO gene protects against hypertension in a Spanish population. Hypertens Res 2012;35:592–6.

[25] Gassó P, Ritter MA, Mas S, Lafuente A. Influence of ABO genotype and phenotype on angiotensin-converting enzyme plasma activity. J Renin Angiotensin Aldosterone Syst 2014;15:580–4.

[26] Seedat Y, Rayner B, Veriava Y. South African hypertension practice guideline 2014. Cardiovasc J Afr 2014;25:288–94.

[27] Lahiri DK, Nurnberger J. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991;19:5444.

[28] PharmGKB [Internet]. PharmGKB. Available at: https://www.pharmgkb.org/. Accessed June 11, 2020.

[29] Ensembl genome browser 100 [Internet]. Available at: https://www.ensembl.org/index.html. Accessed June 7, 2020.

[30] Flaten HK, Monte AA. The pharmacogenomic and metabolomic predictors of ACE inhibitor and angiotensin II receptor blocker effectiveness and safety. Cardiovasc Drugs Therapy 2017;31:471–82.

[31] Ndebele | South African History Online [Internet]. Available at: https://www.sahistory.org.za/article/ndebele. Accessed November 1, 2020.

[32] Lane A, Scoodyall H, Arndt S, et al. Genetic substructure in South African Bantu-speakers: evidence from autosomal DNA and Y-chromosome studies. Am J Phys Anthropol 2002;119:175–85.

[33] Chauhan M, Patel J, Ahmad F. Ramipril. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Available at: http://www.ncbi.nlm.nih.gov/books/NBK537119/. Accessed November 1, 2020.

[34] Holmes DI, Zachary I. The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. Genome Biol 2003; 6:209.

[35] Golas C, Charalabopoulos A, Stagkas D, Charalabopoulos K, Batistatou A. The kinin system - bradykinin: biological effects and clinical implications. Multiple role of the kinin system - bradykinin. Hippokratia 2007;11:124–9.

[36] Parati G, Ochoa JE, Bilo G. Blood pressure variability, cardiovascular risk, and risk for renal disease progression. Curr Hypertens Rep 2012;14:421–31.

[37] Andalib S, Lattanzi S, Di Napoli M, et al. Blood pressure variability: a new predicting factor for clinical outcomes of intracerebral hemorrhage. J Stroke Cerebrovasc Dis 2020;29:105340.