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

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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; $P = .0005$) 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

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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 3 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 is not applicable for this paper.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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dimensionality reduction, NO = nitric oxide, NOS3 = nitric oxide synthase, SNP = single nucleotide polymorphism, VEGF = vascular endothelial growth factor.

Keywords: enalapril, gene-gene interaction, pharmacogenomics, single nucleotide polymorphisms, uncontrolled hypertension

1. 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 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.[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.

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.[5–9] 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.[10–12] It exerts its BP 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 BP 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 *VEGFA* gene, which encodes the VEGF protein. On the other hand rs1042714 and rs2070744 are found on the *ADRB2* gene and *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 (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 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 mm Hg) and uncontrolled (BP ≥140/90 mm Hg).[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 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).

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] 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 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 (GenAlEx) 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 x^2 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 mm Hg) (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 x^2-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

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

BMI = body mass index.

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 2

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

		Ethnic groups			Gender		Age		
DbSNP	Gene	Zulu (n; %)	Swati (n; %)	Xhosa (n; %)	Male (n; %)	Female (n; %)	<55 yrs	55–65 yrs	>65 yrs
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)

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.

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.

	BP			Prescription			
SNP	Controlled (n; %)	Uncontrolled (n; %)	*P* value	Enalapril + 1 (n; %)	Enalapri + 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.

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 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				
Genotypes				
GG			–	
CC	1		1	
GC	2.41 (1.17–4.94)	0.016	2.31 (1.02–5.23)	**.044**
Alleles				
G	1		1	
C	0.84 (0.37–1.72)	0.574	0.94 (0.40–2.26)	.903
rs1799722				
Genotypes				
CC	1		1	
TT	0.85 (0.35–2.02)	0.729	0.97 (0.37–2.56)	.960
CT	1.90 (0.94–4.02)	0.071	2.74 (1.19–6.28)	**.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)	.582
TC	0.61 (0.22–1.68)	0.346	0.76 (0.22–2.56)	.667
Alleles				
C	1		1	
T	0.73 (0.33–1.61)	0.442	0.69 (0.30–1.58)	.386
rs495828				
GG	1		1	
TT	2.84 (0.58–13.91)	0.197	2.10 (0.36–12.10)	.403
TG	1.40 (0.50–3.19)	0.512	1.26 (0.42–3.77)	.479
Alleles				
G			1	
T	1.15 (0.49–2.72)	0.734	0.86 (0.32–2.29)	.772
rs699947				
AA			1	
CC	0.93 (0.33–2.59)	0.895	1.06 (0.31–3.56)	.923
CA	1.21 (0.36–4.01)	0.750	1.79 (0.44–7.18)	.407
Alleles				
A	1		1	
C	0.43 (0.17–1.03)	0.881	0.37 (0.15–0.94)	**.037**

CI = confidence interval, SNP = single nucleotide polymorphism.

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	.3333
NOS3_rs2070744, VEGFA_rs699947	0.6356	0.5711	09/10	.6251
NOS3_rs2070744, ABO_ rs495828, VEGFA_ rs699947	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.

culturally, linguistically, and genetically diverse, however; under-represented in previous drug association studies.[32] It is possible that the 4 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 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.[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 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 BP 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.

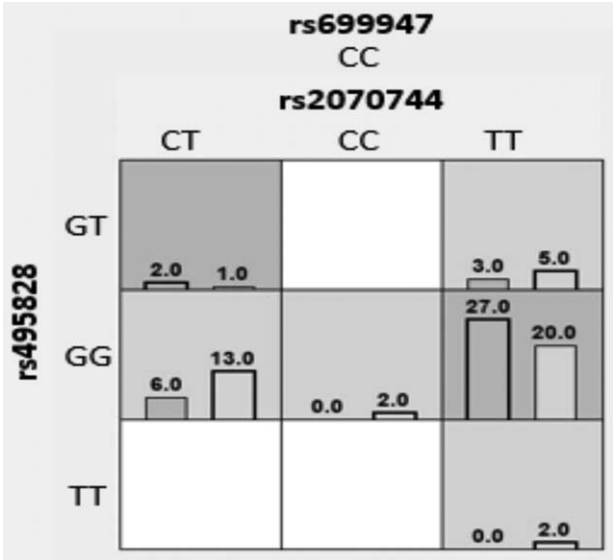

Figure 1. The best MDR model of interaction among ABO rs495828, NOS3 rs2070744, and VEGFA rs699947. The distributions of controlled (left bars) and uncontrolled (right bars) are illustrated for each combination of genotypes. Each cell represents genotype combinations. Dark grey cells represent genotype combinations implicated in enalapril treatment outcome. MDR = multifactor dimensionality reduction, ABO = histo-blood group ABO, NOS3 = nitric oxide synthase, VEGFA = Vascular endothelial growth factor A.

VEGF 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 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 20 mg/day enalapril.[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.[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.[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 BP response in a large African cohort need to be conducted, in order to assess its suitability as a marker for enalapril treatment 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.[16] Also, Oliveira-Paula et al[20] 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 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.

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,[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 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.[36,37] 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.

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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.

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