Variants in Genes Involved in Functional Pathways Associated with Hypertension in African Americans

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Abstract

Essential hypertension (HBP) is a complex trait with a substantial heritable component. The purpose of this study was to determine if variants in the G-protein coupled receptor Kinase-4 (*GRK4*), nitric oxide synthase-3 (*NOS3*), or angiotensin converting enzyme (*ACE*) genes are associated singly or through complex interactions, with HBP in African Americans aged 18–49 years. TaqMan Assays were used for genotyping the *GRK4* and *NOS3* variants. The *ACE* I/D variant was obtained by polymerase chain reaction and electrophoresis. Allelic association tests were performed for the five markers using PLINK. Logistic regression models were fitted to investigate associations between HBP status and the genetic markers. Multilocus analyses were also conducted. The study included 173 hypertensives and 239 normotensives, with stratification into obese and nonobese groups. The *GRK4* A486V variant was negatively associated with HBP in the nonobese group ($p = 0.048$). The TT/CT genotype of *GRK4* A486V was associated with decreased risk for HBP relative to the CC genotype after adjusting for age, sex, and body mass index ($p = 0.028$). Individuals having at least one *NOS3* A allele and *GRK4* R65L genotype GG had odds of HBP of 2.97 relative to GG homozygotes for *NOS3* and *GRK4* R65L. These results show very modest effects and do not fully replicate previous studies. Clin Trans Sci 2010; Volume 3: 279–286

Keywords: hypertension, African Americans, genetic variants, *GRK4*, *ACE*, *NOS3*

Introduction

Among African Americans, hypertension (HBP) is more prevalent, begins at a younger age, is more severe, and causes more target organ damage when compared to other ethnic groups.[1–3] The heritability of HBP is estimated to be between 30% and 50%.[4] In contrast to the monogenic forms of HBP, essential HBP is a complex disorder with an etiology that includes multiple contributory genetic and environmental factors. Several reports describe specific genes that are associated with HBP[5]; as well as genes in functional pathways that are involved in blood pressure (BP) regulation.[6,7]

A leading characteristic of essential HBP is salt sensitivity and over 75% of hypertensive African Americans are salt sensitive.[8,9] Dopamine contributes to sodium balance by facilitating natriuresis through activation of the dopamine receptors (D1) on the renal proximal tubules. G-protein coupled receptor kinase 4 (*GRK4*) is involved in the desensitization of the D1 receptor. Variants in the *GRK4* gene may contribute to sodium retention and HBP through inactivation of the D1 receptor.[4,9]

Endothelial dysfunction, with inappropriate vasoconstriction, is caused by alterations in the nitric oxide (NO) pathway and can be detected early in the development of cardiovascular disease. Endothelial nitric oxide synthase (NOS) is responsible for the production of NO by the vascular endothelial cells. Attenuation in NO synthesis is associated with significant increases in BP.[10,11] In African Americans there is a decrease in NO availability in the endothelium, despite increased NOS activity, which could explain in part the racial disparities in cardiovascular disease prevalence and severity.[12,13] Associations of several *NOS3* variants with HBP have been reported in different populations, but with inconsistent results.[14]

The renin–angiotensin–aldosterone system is involved in both adjustments in vascular tone and sodium–water balance. Lower levels of renin and aldosterone have been demonstrated in salt-sensitive individuals[15,16] and low renin levels are characteristic of hypertensive African Americans.[17] Several candidate genes in the renin–angiotensin–aldosterone system, including angiotensin converting enzyme (ACE) have also been studied for associations with HBP.[10,18–20]

Association studies between HBP and gene variants in African Americans are scarce although there are data showing a significant relationship between HBP and *GRK4 R65L* and *ACE I/D* variants in a population of Africans.[21] An association between susceptibility to ischemic stroke and the 922 A/G variant of NOS3 was also described in a cohort of young African Americans when compared with their Caucasians counterparts.[22] Therefore, the purpose of this study was to determine if variants in the *GRK4* (R65L, A142V, A468V), *NOS3* (–922A/G), or *ACE* (I/D) genes are associated directly or through complex interaction effects, with HBP in a cohort of African American young adults.

Methods

Subjects

The participants were all African Americans between the ages of 18 and 49 years and living in urban Philadelphia. All protocols were approved by the Institutional Review Board of Thomas Jefferson University and written informed consent was obtained from each participant at enrollment. Participants were stratified according to BP status (hypertensive or normotensive) and obesity status (obese or nonobese). Exclusion criteria included secondary HBP, history of diabetes, renal disease, heart failure, autoimmune disease, sickle cell anemia, or endocrine disorders. Individuals of Caribbean descent or pregnant were excluded. BP measurements were obtained by trained research staff according to the American Heart Association protocol.[23] BP was measured, in the seated position, by auscultation. The average of eight separate BP measurements obtained at two separate visits (four measurements at each visit) was used to define BP status. HBP was defined as an average BP ≥140 mmHg systolic and/or ≥90 mmHg diastolic. Participants taking antihypertensive medications were classified

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	All ($n = 412$)	NBP ($n = 239$)	HBP ($n = 173$)	Unadjusted OR (95% CI)	p-value	Adjusted† OR (95% CI)	p-value
Age (years), mean ± S.D.	38 ± 7	36 ± 8	40 ± 6				
Age (years), *n* (%)							
<30	64 (16)	54 (23)	10 (6)	1.00*		1.00*	
30–39	134 (33)	89 (37)	45 (26)	2.73 (1.27–5.86)	0.01	2.43 (1.12–5.28)	0.03
≥40	214 (52)	96 (40)	118 (68)	6.64 (3.21–13.72)	<0.001	5.84 (2.78–12.29)	<0.001
Sex, *n* (%)							
Female	203 (49)	130 (54)	73 (42)	1.00*		1.00*	
Male	209 (51)	109 (46)	100 (58)	1.63 (1.10–2.43)	0.02	1.75 (1.12–2.74)	0.01
BMI (kg/m^2),							
Mean ± S.D.	31.5 ± 8.0	31.3 ± 8.4	32.1 ± 7.3				
BMI group, *n* (%)							
Normal weight	86 (21)	62 (26)	24 (14)	1.00*		1.00*	
Overweight	119 (29)	61 (26)	58 (34)	2.46 (1.36–4.44)	0.003	2.05 (1.09–3.84)	0.03
Obese	207 (50)	116 (49)	91 (53)	2.03 (1.18–3.50)	0.01	2.49 (1.36–4.57)	0.003
Systolic BP							
(SBP, mmHg) mean ± S.D.	121 ± 16	114 ± 9	131 ± 19				
Diastolic BP							
(DBP, mmHg) mean ± S.D.	75 ± 12	70 ± 7	82 ± 13				

95% CI: 95% confidence interval; * = reference category *p*-values are based on the chi-square test; † = adjusted for the other two factors from among age, sex, and BMI.

Table 1. Study participant characteristics, by BP group.

as hypertensive. Normal BP (NBP) was defined as average BP <130 mmHg systolic and <80 mmHg diastolic. Anthropometric measurements and a blood sample were obtained. Body mass index (BMI) was calculated from the height and weight measurements. Obesity was defined as BMI ≥30 kg/m^2.

DNA isolation and genotyping

The genomic sequences for three *GRK4* variants (*GRK4* R65L, *GRK4* A486V, *GRK4* A142V), one *NOS3* variant (−922A/G), and the *ACE* insertion/deletion polymorphism were obtained using previously described methods[21,24] and from the NCBI Entrez website (http://ncbi.nlm.nih.gov/). Genomic DNA was isolated from peripheral blood lymphocytes by Chemagic Magnetic Separation Module I (Chemagen Biopolymer-Technologie AG, Baesweiler, Germany). TaqMan Allelic Discrimination Assays-on-Demand were used for *GRK4* and *NOS3* SNP detection (Applied Biosystems, Foster City, CA, USA) as previously described.[24] Genotypes were determined by ABI 7900HT (Applied Biosystems, Carlsbad, CA, USA) automated caller with quality value of 95% or higher using the Sequence Detection Software version 2.3. For the amplification of the *ACE* genotype the PCR protocol described by Rigat[25] was used with a second PCR for the DD genotype to avoid mistyping of heterozygotes.[26] The second PCR was different from the initial published protocol, as dimethylsulfoxide was not included, and a new sense primer from the 5′ end of the insertion sequence was included along with the standard antisense primer. The cycling conditions were similar to the initial PCR except for an annealing temperature of 61°C. The PCR products were analyzed on DNA 1000 Lab Chips (Agilent Technologies, Palo Alto, CA, USA) by electrophoresis using the Agilent 2100 Bioanalyzer (Agilent Technologies).

Statistical analysis

The association of age (<30, 30–39, ≥40), sex (male, female), and BMI (<25, 25–29, ≥30) with HBP status was examined using a chi-square test. A multivariable logistic regression model for HBP status that included age, sex, and BMI was fitted to determine whether all three factors should be included as covariates in genetic association models. Odds ratios (ORs) and associated 95% confidence intervals were estimated.

Genotype and allele frequency distributions were examined and Hardy–Weinberg equilibrium tests were conducted. Allelic association tests were conducted for each marker using PLINK software version 1.06. The criterion for significance was set at $p < 0.05$ for the allelic association tests as well as for the remainder of association analyses that were conducted using SAS software version 9.2. Reported *p*-values are not adjusted for multiple hypothesis tests.

Logistic regression models were fitted to investigate crude and adjusted associations between HBP status and each of the genetic variants assuming in turn, general, dominant, and recessive genetic models; the adjusted models included age, sex, and BMI. Possible effect modification due to age, sex, or BMI was tested by adding a marker × (age, sex, or BMI) interaction effect to the adjusted logistic regression model. ORs and 95% confidence intervals were estimated using major allele homozygotes as the reference group. Although exact tests were used to analyze sparse data where possible, exact methods could not be used to accommodate the number of covariates in the adjusted models, and the analyses were not conducted. The additive model was also tested for the *ACE* marker. For the *GRK4* A486V marker, an allelic association test was also conducted by fitting a logistic regression model that included the covariates age, sex, and BMI.

Multilocus analyses for all marker pairs were also conducted by fitting logistic regression models for two markers at a time, adjusted for age, sex, and BMI—without any interaction effects. These analyses investigated dominant–dominant models, dominant–recessive models, and recessive–recessive models. The logistic regression analysis was then repeated including a marker × marker interaction effect in the model.

Results

Study group characteristics

Genotypes were determined for 479 participants. Of these, 67 were excluded from data analysis due to an average BP in the range 130–139/80–89 mmHg. Thus, the analyses focused on 412 participants, who were classified as NBP ($n = 239$) or HBP ($n = 173$). The clinical characteristics of the NBP and HBP groups are described in *Table 1*. While relatively young, the HBP group was older than the NBP group. There were also more males, and more overweight and obese participants within the HBP sample. The mean BMI was in the obese range in both groups. The mean BP in the HBP group is lower than expected as patients on antihypertensive medication were included in this group. Age, sex, and BMI showed a significant association with HBP status.

Genotypes

Genotypes were available for all five markers for 320 (78%) of the study participants; 51 (12%) participants had genotypes for four, and 35 (9%) had genotypes for three markers. The three *GRK4* variants were evaluated for linkage disequilibrium (LD) using both our data panel and the HapMap database. The markers *GRK4* R65L and *GRK4* A142V exhibited moderate LD based on both the HapMap (normalized disequilibrium measure, $D' = 0.85$) and our study sample ($D' = 0.74$). *GRK4* A486V did not exhibit LD with the other two *GRK4* markers based on either the HapMap ($D' = 0.51, 0.53$) or our study sample ($D' = 0.41, 0.56$).

Single-locus analyses

Data on allele and genotype frequency according to BP and BMI are summarized for each of the five markers in *Table 2*. The ORs reflect the odds of the minor allele in the HBP group relative to the NBP group. No significant deviation from the expected genotype distribution was found based on the Hardy–Weinberg equilibrium analysis. The *GRK4* A486V variant showed borderline association with the HBP group ($p = 0.056$, data not shown). When the groups were further stratified including BMI, the association between the *GRK4* A486V variant, and HBP in the HBP-BMI <30 subgroup was significant ($p = 0.048$). Logistic regression analysis was conducted to model the allelic association for this marker and adjust for the effects of sex, age, and BMI. This analysis produced an unadjusted OR of 0.68 ($p = 0.057$) for the T allele in the HBP group relative to the NBP group, which was consistent with the allelic association result in *Table 2*. The adjusted OR was strengthened slightly to 0.64 ($p = 0.043$). Because the sex effect was not significant, a final model without sex was fitted which resulted in a negligible change (OR = 0.64, $p = 0.038$).

Further analyses of the association between the five markers and BP group status were conducted to examine the effects of sex, age, and BMI. Results from these analyses are summarized in *Tables 3* through *5*.

Table 3 presents the logistic regression results for the three *GRK4* variants obtained from general, dominant, and recessive models. There is some indication that having at least one T allele may be protective. The TT/CT genotypes are associated with almost half the odds of HBP relative to the CC genotype (adjusted p-value in the dominant model = 0.03). No significant association was found between the *GRK4* R65L and *GRK4* A142V variants and HBP status. Additionally, no significant variant × covariate interaction effects were identified for any of the *GRK4* variants.

Table 4 presents the results for the *ACE* I/D polymorphism. Although the general model appeared to reflect an association

GRK4 A486V	NBP, BMI <30	HBP, BMI <30	NBP, BMI ≥ 30	HBP, BMI ≥ 30	Total
CC	68	61	72	67	268
CT	37	16	28	21	102
TT	2	1	3	2	8
f(C)	0.81	0.88	0.83	0.86	0.84
OR (95% CI)*	0.55 (0.30–1.00)		0.82 (.047–1.43)		
p-value	0.05		0.48		
GRK4 R65L					
GG	34	24	26	25	109
GT	60	45	55	45	205
TT	14	9	17	17	57
f(G)	0.59	0.60	0.55	0.55	0.57
OR (95% CI)	0.99 (0.65–1.50)		1.00 (0.66–1.51)		
p-value	0.95		1.00		
GRK4 A142V					
TT	36	22	37	31	126
CT	55	39	56	43	193
CC	17	15	13	12	57
f(T)	0.59	0.55	0.61	0.61	0.59
OR (95% CI)	1.19 (0.78–1.80)		1.01 (0.67–1.53)		
p-value	0.42		0.96		
ACE I/D					
DD	36	29	48	30	143
DI	54	32	48	42	176
II	21	19	16	18	74
f(D)	0.57	0.56	0.64	0.57	0.59
OR (95% CI)	1.02 (0.68–1.54)		1.38 (0.92–2.06)		
p-value	0.92		0.12		
NOS3 intron					
AA	89	61	77	54	281
AG	27	18	34	31	110
GG	2	2	5	1	10
f(A)	0.87	0.86	0.81	0.81	0.84
OR (95% CI)	1.04 (0.58–1.87)		1.01 (0.61–1.68)		
p-value	0.90		0.96		

f( ): frequency of major allele; * = allelic association ORs and *p*-values; OR: odds ratio, represents the odds of the minor allele for HBP relative to NBP.

Table 2. Summary of allele frequencies, genotype distributions, and associations within BP and body size groups.

	All *n*	NBP *n* (%)	HBP *n* (%)	General model				Dominant				Recessive			
				Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted	
				OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
GRK4 A486V ($n = 378$)															
CC	268	140 (67)	128 (76)	1.00*		1.00*		1.00*		1.00*					
CT	102	65 (31)	37 (22)	0.62	0.06†	N/A						1.00*		1.00*	
				(0.38–1.02)				0.63	0.04	0.58	0.03				
TT	8	5 (2)	3 (2)	0.66	0.84†	N/A						0.75	0.74†	N/A	
				(0.10–3.46)				(0.40–0.99)		(0.36–0.94)		(0.11–3.90)			
Global *p*-value					0.13†		N/A		0.04		0.03		0.74†		N/A
GRK4 R65L ($n = 371$)															
GG	109	60 (29)	49 (30)	1.00*		1.00*		1.00*		1.00*					
GT	205	115 (56)	90 (55)	0.96	0.29	0.90	0.68					1.00*		1.00*	
				(0.60–1.53)		(0.55–1.49)		0.97	0.91	0.92	0.75				
TT	57	31 (15)	26 (16)	1.03	0.94	1.01	0.97					1.06	0.85	1.09	0.79
				(0.54–1.96)		(0.51–2.02)		(0.62–1.53)		(0.57–1.50)		(0.60–1.86)		(0.59–1.99)	
Global *p*-value					0.97		0.89		0.91		0.75		0.85		0.79
GRK4 A142V ($n = 376$)															
TT	126	73 (34)	53 (33)	1.00*		1.00*		1.00*		1.00*					
CT	193	111 (52)	82 (51)	1.02	0.51	1.01	0.98					1.00*		1.00*	
				(0.65–1.60)		(0.62–1.64)		1.07	0.78	1.10	0.69				
CC	57	30 (14)	27 (17)	1.24	0.50	1.50	0.24					1.23	0.48	1.50	0.20
				(0.66–2.33)		(0.76–2.97)		(0.69–1.64)		(0.69–1.76)		(0.70–2.16)		(0.81–2.76)	
Global p-value					0.78		0.43		0.78		0.69		0.48		0.20

* = reference category; OR: odds ratio 95% CI: 95% confidence interval; global *p*-value refers to the overall association of the marker with BP group; †p-values are exact and account for sparse data; N/A: not available (the adjusted model could not be not fitted because of the sparse data.

Table 3. *GRK4* markers and BP (normotensive—NBP vs. hypertensive—HBP).

	All *N*	NBP *n* (%)	HBP *n* (%)	General model				Dominant				Recessive			
				Unadjusted		Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted	
				OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
DD	143	84 (38)	59 (35)	1.00*		1.00*		1.00*		1.00*					
ID	176	102 (46)	74 (44)	1.03	0.89	1.33	0.25					1.00*		1.00*	
				(0.66–1.62)		(0.82–2.17)		1.14	0.55	1.42	0.13				
II	74	37 (17)	37 (22)	1.42	0.22	1.64	0.11					1.40	0.20	1.40	0.23
				(0.81–2.50)		(0.89–3.01)		(0.75–1.72)		(0.91–2.23)		(0.84–2.32)		(0.81–2.41)	
Global *p*-value					0.43		0.25		0.55		0.13		0.20		0.23

OR: odds ratio; 95% CI: 95% confidence interval; * = reference category; global *p*-value refers to the overall association of the marker with BP classification.

Table 4. *ACE* insertion-deletion and BP (*n* = 393).

	All *n*	NBP *n* (%)	HBP *n* (%)	General model	Dominant				Recessive						
				Unadjusted	Adjusted		Unadjusted		Adjusted		Unadjusted		Adjusted		
				OR (95% CI)	*p*-value†	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value†	OR (95% CI)	*p*-value
AA	281	166 (71)	115 (69)	1.00*		1.00*		1.00*		1.00*					
AG	110	61 (26)	49 (29)	1.16	0.59	N/A						1.00*		1.00*	
				(0.74–1.81)			1.10		0.65	1.10	0.68				
GG	10	7 (3)	3 (2)	0.62	0.73	N/A						0.59	0.68	N/A	
				(0.16–2.44)				(0.72–1.70)		(0.69–1.75)		(0.15–2.33)			
Global *p*-value					0.60	N/A			0.65		0.68		0.68		N/A
Interaction *p*-values															
w/age group							N/A				0.06				N/A
w/sex							N/A				0.24				N/A
w/BMI group							N/A				0.71				N/A

OR: odds ratio; 95% CI: 95% confidence interval; * = reference category; global *p*-value refers to the overall association of the marker with BP classification; †*p*-values are exact and account for sparse data; N/A: not available (model not fit because of sparse data).

Table 5. *NOS3* intron and BP (*n* = 401).

(having one I allele increased the odds of HBP by approximately 30%, although two I alleles increase the odds by approximately 60%, the overall association between this marker and HBP status was not significant (adjusted $p = 0.25$). However, when an additive model was assumed, the association was borderline significant; each additional I allele increased the odds of HBP by about 29%, $p = 0.10$ (data not shown).

Table 5 presents the results for the *NOS3* marker. Although the dominant model did not suggest an association between the marker and HBP status (adjusted *p* for dominant model = 0.68),

GRK4 R65L	*NOS3*	All *n*	NBP *n*	HBP *n*	Interaction OR (95% CI)	*p*-value
GG	GG	77	47	30	1.00*	
TT/GT	GG	176	99	77	1.28 (0.71–2.39)	0.412
GG	AA/AG	28	12	16	2.97 (1.15–7.71)	0.025
TT/GT	AA/AG	79	43	36	1.31 (0.66–2.58)	0.443

* = reference group; adjusted for age, sex, and BMI.

Table 6. Adjusted ORs from the logistic model that included a *GRK4* R65L × *NOS3* interaction effect ($N = 360$).

there was a suggestion of an interaction effect between this variant and age ($p = 0.06$). The OR for the variant (GG/AG relative to AA) is 0.57 ($p = 0.51$) among participants <30 years of age, 2.44 ($p = 0.03$) among those 30 to 39 years, and 0.77 ($p = 0.41$) among those ≥40 years. Given the explorative nature of this study the U-shaped pattern should be interpreted with caution.

Multilocus analyses

Prior to testing for pairwise marker × marker interaction effects, logistic regression analysis was used to model the main effects of two markers at a time. Logistic regression models that included the pairwise interaction effects were then fitted to test for possible effect modification among the five markers assuming both dominant and recessive models. The *NOS3* and *GRK4* A486V markers did not have sufficient numbers for the recessive model analysis. Interaction effects were tested for dominant × dominant models, dominant × recessive models, and recessive × recessive models, with adjustment for age, sex, and BMI. Typical global *p*-values for the interaction effects were 0.20 and higher. One exception was the interaction effect between the *NOS3* dominant G allele model and the *GRK4* R65L dominant T allele model that was borderline significant with $p = 0.06$. The samples sizes and adjusted ORs for combined *GRK4* R65L and *NOS3* genotypes under this model are displayed in *Table 6*. Among participants with the GG genotype for *GRK4* R65L, the unadjusted odds of HBP among individuals having at least one *NOS3* A allele relative to those with the GG genotype is 2.09 (95% CI: 0.87–5.02). The increase in OR following adjustment is largely due to age and BMI differences among the four genotype combinations. The model adjusted for only age produces an OR of 2.63 (95% CI: 1.04–6.69), while the model adjusted for only BMI results in an OR of 2.43 (95% CI: 1.15–7.71). A model with BMI and age produced an OR of 2.88 (95% CI: 1.11–7.42). The OR increased to 2.97 once the model accounted for age, BMI, and sex. There is almost no increase in the odds of HBP associated with carrying at least one A allele among participants with the TT/GT genotype for *GRK4* R65L, indicating effect modification. Although this *NOS3* × *GRK4* R65L interaction effect ranks highest among the models tested, the *p*-value is only suggestive.

Discussion

Analysis of our data detected a significant association of the *GRK4* A486V variant with HBP in our nonobese sample of young African Americans. The T allele (or V allele if we report the amino acid change) of this variant conferred a protective effect. Because the number of participants with the minor allele was small, this association should be interpreted with caution. In addition, the TT and CT genotypes, in a dominant model were associated with reduced risk for HBP compared to the CC genotype. There was no significant association of either the *GRK4* R65L or A142V variants with HBP in our cohort.

Although data linking specific GRK4 variants to HBP in African Americans is scarce, our study adds to the limited reports on associations between polymorphisms in GRK4 and HBP in African Americans. However our data does not fully replicate other published reports that include variants of the *GRK4* gene in different African American cohorts or other ethnicities: Zhu et al.[27] found a strong interaction between the R65L variant and age for systolic BP in a cohort of African Americans and white twins. Carriers of the L65L allele had the steepest increase of BP with age but this effect was only significant for the older cohort of participants. These results were similar in both ethnic groups. More recently GRK4 variants have been associated with a decreased response to beta-blocker treatment for HBP in African American males.[28] GRK4 variants and HBP have been studied in other ethnic groups with variable results. Wang and Gu showed an association of the A allele of the A486V variant with HBP in Han Chinese,[29,30] Speirs et al. demonstrated an association of the V allele of the A486V variant with HBP in Australians[31] and Bengra et al. demonstrated an association of the A486V variant with HBP in Italians.[32] A metaanalysis of the major studies involving *GRK4* variants and HBP showed that the allele 486V increases the risk for HBP with an OR of 1.5 (95% CI: 1.2–1.9).[33] Other studies have found associations between the A142V variant[34] and HBP in a Japanese population.

An association of *ACE* polymorphism with HBP has been reported in African Americans,[35] and also in other populations including southern Chinese,[20,36] Asian Indians,[37] Italians,[38] and Hispanics.[39] In our cohort of African Americans, there was a suggestion of an association under the additive genetic model, with each additional I allele increasing the risk for HBP by an estimated 29%. However, this effect was not statistically significant given the limited sample size. Moreover, our data suggest that the I allele confers risk. This is in contrast to the results of Duru et al.,[35] who reported that the D allele was more frequent in African Americans with HBP compared with normotensives. In an Asian Indian population Das et al.[37] reported that the DD genotype confers an OR for HBP of 7.4 relative to those carrying at least one I allele. A recent study in a Chinese cohort reported that the *ACE* D allele in a dominant model was associated with HBP.[20] These three studies differ from the results of our study in which we could not demonstrate a significant association of the D allele with HBP in a cohort of relatively young African Americans.

There have been no previous reports on an association between NOS genotypes and HBP in African Americans. In our study, the *NOS3* GG/AG genotypes were associated with HBP but only in the subset that was 30 to 39 years of age. Because the association in older and younger individuals suggested a protective effect and the overall significance was borderline, these results should be interpreted with caution. The association of genetic variants in *NOS3* with HBP in African Americans may be modified by age and this requires further investigation. Associations of polymorphisms in the *NOS3* gene with essential HBP have been investigated in white Europeans,[40] Indians,[41] Japanese,[42,43] Chinese,[44] and renal transplant recipients.[45] Despite some positive associations more recent investigations and a metaanalysis have shown conflicting results.[14,46]

While some significant associations of HBP with variants within single genes have been described, these findings have been modest and difficult to reproduce. The genetic complexity of HBP as a trait most probably involves gene–gene and gene–environmental interaction effects. We attempted to address this issue in our multilocus analysis. When we combined the five variants under the dominant or recessive model and adjusted for age, sex, and BMI, the combination of the *NOS3* variant and *GRK4* R65L under the dominant model detected a borderline association with HBP. African Americans in our study that had at least one *NOS3* A allele and the *GRK4* R65L GG genotype have an OR for HBP of 2.97. These data differ from a study by Williams et al.[21] who reported that, in an African population from Ghana, the combination of *ACE* I/D and *GRK4* R65L was the best model to predict HBP. Alternatively in a haplotype analysis of data from a northern Han Chinese population, Zhu et al.[27] reported that the haplotype 65L-142V-486A was associated with an increased risk of HBP with increasing age.

The size of our cohort was similar or larger than that of other genetic association studies in African Americans. However a limitation of all these studies including ours is the modest sample size, which provides limited power. For a dominant model analysis, the study had 80% power to detect ORs of about 2.0 or greater. For a recessive model analysis, the study had sufficient power to detect ORs of 2.4 or greater for minor allele frequencies of about 40% (e.g., *GRK4* R65L, A142V and *ACE*) and 6.5 or greater for rarer minor allele frequencies of about 15% (e.g., *GRK4* A486V and *NOS3*). For effects of a similar magnitude to those actually observed in our study (e.g., OR of 1.4 for *ACE*), a total sample size of about 2,200 would have been required to have 80% power to detect the association.

As with the most genetic association studies of HBP, the BP phenotype is difficult to verify. HBP can be difficult to confirm due to variability in BP measurements and the effect of age on phenotypic expression. This is especially difficult in a young population such as our study group in which some individuals designated as normotensive may develop HBP as they age. For this reason we excluded participants with BP levels in the prehypertensive range and we also adjusted for age and BMI.

Conclusions

Our study characterized several candidate genes involved in different functional pathways that could contribute to the development of HBP. Although the genetic variants associated with HBP in our cohort do not replicate previous studies, the results showed some evidence that small effects of multiple genes contribute to higher BP in African Americans. Other analytic strategies and larger well-characterized populations may be necessary to detect gene–gene and gene–environment interaction effects.

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Conflict of Interest

The authors report no conflict of interest.

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