SNP-by-fitness and SNP-by-BMI interactions from seven candidate genes and incident hypertension after 20 years of follow-up: The CARDIA Fitness Study

Mark A. Sarzynski, Ph.D.1, Tuomo Rankinen, Ph.D.1, Barbara Sternfeld, Ph.D.2, Myriam Fornage, Ph.D.3, Stephen Sidney, M.D.2, and Claude Bouchard, Ph.D.1

1Human Genomics Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA
2Division of Research, Kaiser Permanente Medical Care Program, Oakland, CA 94611, USA
3Institute of Molecular Medicine, University of Texas Health Science Center, Houston, TX 77030, USA

Abstract

The association of SNPs from seven candidate genes, including genotype-by-baseline fitness and genotype-by-baseline body mass index (BMI) interactions, with incident hypertension over 20 years was investigated in 2663 participants (1301 blacks, 1362 whites) of the Coronary Artery Risk Development in Young Adults Study (CARDIA) Study. Baseline cardiorespiratory fitness was determined from duration of a modified Balke treadmill test. A total of 98 SNPs in blacks and 89 SNPs in whites from seven candidate genes were genotyped. Participants that became hypertensive (295 blacks and 146 whites) had significantly higher blood pressure and BMI (both races), and lower fitness (blacks only) at baseline than those who remained normotensive. Markers at the PPARGC1A and BDKRB2 genes were nominally associated with greater risk of hypertension, while one marker each at the BDKRB2 and NOS3 genes were nominally associated with lower risk. The association of baseline fitness with risk of hypertension was nominally modified by genotype at markers within the ACE, AGT, BDKRB2, and NOS3 genes in blacks and the BDKRB2, EDN1, and PPARGC1A genes in whites. BDKRB2 rs4900318 showed nominal interactions with baseline fitness on the risk of hypertension in both races. The association of baseline BMI with risk of hypertension was nominally modified by GNB3 rs2301339 genotype in whites. None of the above associations were statistically significant after correcting for multiple testing. We found that SNPs in these candidate genes did not modify the association between baseline fitness or BMI and risk of hypertension in CARDIA participants.
INTRODUCTION

It is estimated that approximately 65 million U.S. adults have hypertension, a well-known independent risk factor for cardiovascular disease. Hypertension is viewed as a complex trait caused by the interaction of multiple susceptibility genes with environmental and behavioral factors. Risk factors for hypertension include poor diet, physical inactivity, low cardiorespiratory fitness (hereafter referred to as fitness), overweight and obesity, smoking, and low education level. Prospective epidemiological studies have shown a lower risk of developing hypertension in physically fit compared to unfit individuals. The Coronary Artery Risk Development in Young Adults (CARDIA) Study found that during a 15 year follow-up period, participants with low fitness (< 20th percentile) were 3 times more likely to develop hypertension than participants with high fitness (≥ 60th percentile), and the population attributable risk of developing hypertension due to low fitness was 21%. Furthermore, overweight and obesity are associated with increased risk of developing hypertension. In CARDIA, race- and sex-specific odds ratios (OR) for incident hypertension over 10 years of follow-up associated with a 1-standard deviation (SD) increase in body mass index (BMI) ranged from 1.33 to 1.66 (P≤0.001).

However, some individuals will become hypertensive despite being physically fit and/or having a normal body weight, whereas others who are unfit and/or overweight may have blood pressure values in the normal range. For example, although fitness level was the strongest predictor of hypertension risk in the HYPGENE study, 37% of the subjects in the highest fitness decile became hypertensive and one third of the subjects in the lowest decile remained normotensive during the follow-up period. Similarly, whereas exercise training and weight loss lower blood pressure on average, there are significant inter-individual differences in blood pressure responses to identical interventions. The underlying mechanisms for inter-individual variation in the effects of regular physical activity on blood pressure are still poorly understood, but initial blood pressure level and familial factors are involved. The HERITAGE Family Study has shown that the heritabilities of endurance training–induced changes in hemodynamic phenotypes after completion of a standardized endurance-training program vary between 20 and 40%, and some of the classic hypertension candidate genes affect the training responses. Thus, identifying the sources of variation in blood pressure is critical for a better understanding of the pathophysiological processes leading to hypertension and the mechanisms by which higher levels of fitness or normal weight may protect against hypertension.

Several cross-sectional studies have found significant single nucleotide polymorphism (SNP)-by-physical activity and SNP-by-BMI interactions on blood pressure phenotypes using the candidate gene approach. However, similar data on the contribution of genetic variation to the antihypertensive effect of increased fitness levels over time are scarce, mainly because of the lack of suitable data sets to examine such questions. The CARDIA
study is a longitudinal study of black and white young adults that includes measures of symptom-limited exercise test duration at three time points over 20 years of follow-up. Thus, the CARDIA cohort represents an excellent resource to examine hypotheses regarding the genetic basis of hypertension while taking fitness and BMI levels into account. The purpose of the present study was to examine the association of SNPs, as well as SNP-by-baseline fitness and SNP-by-baseline BMI interactions, in seven candidate genes with risk of incident hypertension after 20 years of follow-up in the CARDIA Fitness Study.

METHODS

Study population

Details of recruitment, study design, and methods of the CARDIA study have been published elsewhere. The initial examination included 5115 black and white men and women aged 18-30 years. Participants were recruited to represent proportionate racial, sex, age, and education groups from four U.S. communities: Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. Six sequential examinations have been conducted from the time of initiation of the study in 1985-86 through year 20 (2005-06). Retention rates declined from 90% to 72% across examinations. All participants provided written informed consent, and institutional review boards from each field center and the coordinating center approved the study annually.

Genotype data were obtained on 4244 individuals who participated in the baseline examination. To be included in analyses of 20 year incident hypertension, participants had to have measurements of systolic and diastolic blood pressure (SBP and DBP, respectively) from both the baseline and year 20 examinations, be free of hypertension and not be taking any blood pressure medication at baseline, and free of other chronic diseases and not pregnant at both examinations. At baseline, we excluded participants that did not have data for treadmill time or BMI and who reported having or were unsure of their status regarding the following health risks: hypertension/blood pressure medication, heart problems, diabetes, cancer, and women who were pregnant (total excluded for these reasons: n=967). To minimize potential classification errors, parents’ race (reported by the participants) had to match that of the participant (n=132 excluded). This resulted in 3145 (1490 blacks, 1655 whites) healthy participants at baseline. Exclusions at year 20 included participants reporting heart problems, cancer, diabetes, HIV, women who were pregnant, and not sure of their hypertension/blood pressure medication status (total excluded for these reasons: n=482). This resulted in a total cohort of 2663 participants (1301 blacks, 1362 whites) being included in analyses of incident hypertension over 20 years of follow-up. There were small baseline differences between participants included in the analyses and those excluded. Participants included in the analyses were younger (24.6 vs 25.5 years old, P<0.0001), had lower BMI (24.0 vs 25.4 kg/m², P<0.0001), had lower blood pressure (SBP: 109.1 vs 112.8 mmHg, P<0.0001); DBP: 67.5 vs 70.8 mmHg, P<0.0001), and higher treadmill time (10.04 vs 9.31 min., P<0.0001) at baseline compared to those excluded.
Definition of Hypertension

Hypertension was defined at baseline as resting SBP ≥140 mmHg or resting DBP ≥90 mmHg, or positive responses to the questions, “Has a doctor or nurse ever said you had high blood pressure?” or “Have you ever taken medication for high blood pressure?”

Hypertension at the year 20 examination was defined similarly except the last question was phrased, “Are you (currently) taking medications for high blood pressure?” The hypertension endpoint is based on blood pressure cut points used in the seventh report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. Thus, hypertensive participants are defined as those who were normotensive at baseline but developed hypertension over 20 years of follow-up, whereas normotensive participants are those who remained free of hypertension during the 20 year follow-up period. Of included incident hypertension cases (n=441 total), 348 (78.9%) were confirmed on the basis of hypertension diagnosis and medication, 17 (3.9%) had elevated SBP, 38 (8.6%) had elevated DBP, and 38 (8.6%) had both elevated SBP and DBP.

Data Collection

The Coordinating Center and the CARDIA Quality Control Commission monitored quality of data collection. The CARDIA Study staff was centrally trained and certified, and followed standardized protocols across study centers and examinations. Participants were asked to fast for at least 12 hours and to not smoke or engage in heavy physical activity for at least 2 hours prior to the examination.

Blood Pressure—Blood pressure was measured using a Hawksley random zero sphygmomanometer (WA Baum Company, Copaigue, NY) at baseline and the Omron HEM-907XL digital blood pressure monitor (Omron Healthcare, Kyoto, Japan) at year 20. Each participant sat in a quiet room for 5-min prior to having three blood pressure measurements taken from the right arm at 1-min intervals by trained and certified technicians. Systolic and diastolic pressures were recorded as Phase I and Phase V Korotkoff sounds. The average of the second and third measurements was used for analyses.

Fitness assessment—Symptom-limited graded exercise treadmill testing was performed at baseline according to a modified Balke protocol. The test consisted of up to nine 2-min stages of progressively increasing difficulty. Stage 1 was at 3.0 mph and 2% grade, stages 2-6 were at 3.4 mph with grade beginning at 6% and increasing by 4% each stage, stages 7-8 were at 4.2 mph and 22% and 25% grade respectively, and stage 9 was at 5.6 mph and 25% grade. The first six stages could generally be performed by walking. The exercise test consisted primarily of walking to facilitate performance by those unaccustomed to jogging and to allow for easier replication during future follow-up exams. Fitness was defined as the duration of the treadmill test in minutes.

Other measurements—Body weight (light clothing) was measured to the nearest 0.23 kg using a beam balance scale. Height without shoes was measured to the nearest 0.5 cm using a vertically mounted ruler and a metal carpenter’s square. BMI was calculated as weight (kg) divided by height squared (m²). Each participant’s age, race, and sex were self-reported during the recruitment phase and verified during the baseline clinic visit. Standardized
questionnaires were used to obtain data on sociodemographic risk factors such as years of education (highest number of years of school completed) and smoking status (current, former, and never smoker), as well as diagnosis and treatment of hypertension, diabetes, and other conditions.

**SNP selection**

For the present study, we included SNPs from seven established blood pressure and/or fitness candidate genes: angiotensin converting enzyme (ACE), angiotensinogen (AGT), bradykinin β2 receptor (BDKRB2), endothelin-1 (EDN1), guanine nucleotide binding protein beta-3 subunit (GNB3), endothelial nitric oxide synthase 3 (NOS3), and peroxisome proliferative activated receptor gamma coactivator 1 alpha (PPARGC1A). TagSNPs within these genes with a minor allele frequency (MAF) of greater than 0.05 were derived using the Haploview Program based on two sources of SNPs: the Caucasian (CEU) and Yoruban (YRI) population from the International HapMap project. The algorithm used for SNP selection was Haploview’s implementation of the Broad Institute’s Tagger software, with the R squared cut off for Tagger set to 0.8 and the LOD threshold to 2. In addition, Tagger was used in aggressive multi-marker mode. All tagSNPs selected by Tagger for the CEU population were included in the SNP panel. TagSNPs that were not in blocks, or only tagged themselves in the YRI population were not included. Nonsynonymous SNPs with a MAF >0.05 were also included. The final SNP set included 101 SNPs in blacks and 90 SNPs in whites with MAF >0.05 and Hardy-Weinberg equilibrium (HWE) p ≥ 0.0008 in the CARDIA cohort. The selected SNPs capture 50-91% of the common genetic variance reported for the YRI population and 60-100% for the CEU population according to the International HapMap project Phase 2 release 24 data (Supplementary Table S1). One SNP in EDN1 and two SNPs in PPARGC1A in blacks and one SNP each in NOS3 and PPARGC1A and two SNPs in AGT in whites were genotyped that were not reported in HapMap and were not in LD with HapMap tagSNPs and therefore potentially capture part of the remaining genetic variation for these genes in the YRI and CEU populations.

**Genotyping**

Genotyping of the polymorphisms was performed using a two step approach. SNPs were multiplexed in a reaction using the iPLEX MassARRAY genotyping system (Sequenom, Inc.; San Diego, CA). SNPs that did not perform well on this platform were then genotyped using TaqMan Pre-Validated SNP assays (Applied Biosystems; Foster City, CA). Details for PCR conditions and primer sequences are available on request. Genotyping was successfully performed in 82% of the original SNP set (n=354 SNPs from 17 genes) of the CARDIA Fitness Study. Replicate samples (n=206) were randomly dispersed throughout the genotyping plate set. Only SNPs that had a minimum concordance of 99% were used for further analyses.

**Statistical Analysis**

All statistical analyses were performed with SAS version 9.1 (SAS Institute Inc, Cary, NC). Means and SD were computed for all descriptive characteristics by race. Differences in continuous and categorical variables between incident hypertensives and normotensives by
race were assessed using t-tests and chi-square tests, respectively. HWE was tested in the included SNPs of each race by comparing observed genotype frequencies to expected frequencies using the ALLELE procedure in SAS. The pair-wise linkage disequilibrium (LD) among the SNPs was assessed using the ldmax program available in the GOLD software package. Logistic regression modeling was used to assess the contribution of age, sex, BMI, fitness, DBP, SBP, smoking, follow-up time, and education to the risk of incident hypertension by race. All variables were based on baseline values except for follow-up time. ORs and 95% confidence intervals (CI) were calculated for each variable in the multivariate models.

Logistic regression modeling was used to test the contributions of SNPs, as well as SNP-by-baseline fitness and SNP-by-baseline BMI interactions to the risk of incident hypertension. In the basic models testing the association of SNPs with hypertension, each SNP was entered individually into the model with baseline values of age, sex, fitness, BMI, DBP, and SBP included as covariates. BMI was log transformed because of its skewed distribution. The common allele homozygotes served as the reference group in the basic models.

Interactions between SNPs and baseline fitness (dichotomous trait: high/low) and SNPs and baseline BMI (dichotomous trait: high/low) were tested by including interaction terms (SNP*fitness group; SNP*BMI group) in the basic logistic regression models. In the interaction models, baseline fitness and BMI values were dichotomized into low- and high-groups based on race- and sex-specific median cut points. Specifically, low- and high-fitness groups based on race- and sex-specific median cut points of baseline treadmill time (blacks: males-11.5 min., females-7.5 min.; whites: males-12.6 min., females-9.3 min.), and low- and high-BMI groups based on race- and sex-specific median cut points of baseline BMI (blacks: males- 23.5 kg/m$^2$, females- 23.7 kg/m$^2$; whites: males- 23.6 kg/m$^2$, females- 21.8 kg/m$^2$) were used. Baseline values of age, sex, fitness (BMI interaction model), BMI (fitness interaction model), DBP, and SBP were included as covariates in the interaction models.

The interaction models tested for the main effect of each SNP, as well as the SNP interaction term (SNP*fitness group or SNP*BMI group) in the total sample stratified by race. Post-hoc analyses of nominally significant interactions were performed using race-specific analyses stratified by baseline fitness or BMI group, with no further adjustment for fitness or BMI. The common allele homozygotes in the low-fitness or low-BMI group served as the reference group (OR=1.0) in the interaction models.

Participants were grouped by minor allele carrier status for SNPs with MAF <10% showing nominally significant (p<0.05) associations with incident hypertension. Minor allele carrier status was also used when the cell size for a genotype-by-hypertension or genotype-by-hypertension-by fitness or BMI group was less than 10. Since multiple SNPs were used in the association analyses, we applied a multiple testing correction proposed by Nyholt. Briefly, the method uses spectral decomposition of matrices of pairwise LDs ($r$) to estimate variance of eigenvalues. The effective number of independent SNPs ($M_{\text{eff}}$) at each gene can be calculated based on the ratio of observed eigenvalue variance ($\hat{\lambda}_{\text{obs}}$) and its maximum (M): $M_{\text{eff}} = 1 + (M-1) \left(1 - \frac{\text{Var} \, \hat{\lambda}_{\text{obs}}}{M}\right)$. This procedure was performed separately for each gene locus and the effective number of SNPs from each locus was then added over all loci to derive the total number of effective SNPs. The total number of effective SNPs can then be used to adjust the standard α level (e.g., 5%) using a standard Bonferroni correction. Since
LD between the SNPs differed between black and white participants, the total effective number of SNPs was also different (98 in blacks, 64 in whites). Thus, in our study the corrected threshold for statistical significance was set to \( P<0.0005 \) in black participants and \( P<0.0008 \) in white participants. Post-hoc power analyses were performed using QUANTO version 1.2.4 (http://hydra.usc.edu/gxe).\(^{31}\)

**Results**

Table 1 shows the baseline characteristics of participants by race and 20 year incident hypertension status. In black participants, hypertensives (n=295) were significantly older, had higher BMI and resting blood pressure (both SBP and DBP), and were less fit at baseline than normotensives. In whites, a higher proportion of hypertensives were men, whereas a higher proportion of normotensives were women. Furthermore, in white participants, hypertensives (n=146) were significantly heavier and had higher BMI and resting blood pressure (both SBP and DBP) at baseline compared to normotensives. Overall, a higher proportion of subjects with low fitness at baseline were hypertensive (blacks: 26\% of low-fit group, whites: 12\%) compared to subjects that were in the high-fitness group at baseline (blacks: 20\% of high-fit group, whites: 9\%), with the difference being significant in blacks only (\( P=0.01 \)). Similarly, a higher proportion of hypertensives were in the high-BMI compared to low-BMI group at baseline in both races (blacks: 27\% of high-BMI group compared to 18\% of low-BMI group, \( P<0.0001 \); whites: 14\% of high-BMI group vs 8\% of low-BMI group, \( P=0.001 \)).

Results of the multivariate logistic regression models for the risk of incident hypertension by race are shown in Table 2. In both races, baseline SBP showed the strongest association with the risk of hypertension, followed by baseline DBP. Greater baseline fitness level was associated with lower risk of hypertension in both races, whereas no other variables were significantly associated with incident hypertension. The allele frequencies, HWE, and the pairwise LD among all included SNPs are summarized by race in supplementary Tables S2-S3. The results for the association of SNPs with incident hypertension and SNP-by-baseline fitness and SNP-by-baseline BMI interactions for all SNPs are summarized by race in supplementary Tables S4-S5.

**Association of SNPs with incident hypertension**

The nominally significant associations of SNPs with incident hypertension by race are presented in Table 3. SNPs from four genes (AGT, ACE, EDN1, and GNB3) were not associated with incident hypertension in either race. The minor alleles of three PPARG1A SNPs in blacks and two PPARG1A and three BDKRB2 SNPs in whites were nominally associated (\( p<0.05 \)) with greater risk of incident hypertension (Table 3). In contrast, the minor alleles at the BDKRB2 rs8016905 locus in blacks and the NOS3 rs1808593 locus in whites were nominally associated with a lower risk of incident hypertension. None of these associations were statistically significant when taking into account the race-specific multiple testing corrected thresholds for statistical significance.
SNP-by-Fitness interactions and incident hypertension

There was no evidence of SNP-by-fitness interactions on the risk of incident hypertension in either race for SNPs in the GNB3 gene locus. The BDKRB2 rs4900318 marker showed nominally significant interactions with baseline fitness level on the risk of incident hypertension in both black and white participants (Table 4), although the interaction patterns and minor alleles differed by race (Figure 1). In blacks, participants who were high-fit at baseline and homozygous for the common allele (A/A) of the BDKRB2 rs4900318 marker had a 44% lower risk of incident hypertension, while no association was observed in low-fit participants (Figure 1a). In whites, minor allele homozygotes (A/A) of the BDKRB2 rs4900318 marker had an over 3-fold greater risk of incident hypertension only in participants that were high-fit at baseline (Figure 1b).

Blacks—One SNP each at the AGT, NOS3, and ACE genes and three SNPs at the BDKRB2 gene showed nominally significant interactions with baseline fitness level on the risk of incident hypertension in black participants (Table 4). High-fit participants carrying the minor allele of the ACE rs4303 marker had a 55% lower risk of incident hypertension, while no association was observed in participants that were low-fit at baseline (Supplementary Figure S1). Minor allele homozygotes at the NOS3 rs3918188 marker had an almost two-fold greater risk of incident hypertension only in participants low-fit at baseline, while no association was observed in high-fit participants (Supplementary Figure S2). Within the BDKRB2 gene, minor allele homozygotes of the rs945039 marker had a 64% lower risk of incident hypertension only in high-fit participants, whereas minor allele homozygotes of the rs4905474 marker had two-fold greater risk of incident hypertension only in high-fit participants (Supplementary Figure S3).

Whites—Two SNPs each at the BDKRB2 and PPARGC1A genes and the EDN1 rs2071943 marker showed nominally significant interactions with baseline fitness level on the risk of incident hypertension in white participants (Table 4). Minor allele carriers of the PPARGC1A rs2932965 marker had a 55% lower risk of incident hypertension only in high-fit participants, whereas common allele homozygotes of the PPARGC1A rs6838600 marker had a 45% lower risk of incident hypertension only in high-fit participants (Supplementary Figure S4). Additional figures showing nominal SNP-by-fitness interactions can be found in Supplementary Figures S5-S7. None of the SNP-by-fitness interaction terms in either race were statistically significant when taking into account the race-specific multiple testing corrected thresholds for statistical significance.

SNP-by-BMI interactions and incident hypertension

The SNP-by-BMI interaction term was nominally significant for the GNB3 rs2301339 marker in white participants (P=0.006). In white participants, minor allele carriers of the GNB3 rs2301339 marker showed a 64% lower risk of incident hypertension compared to common allele homozygotes in the low-baseline BMI group, while genotype was not associated with incident hypertension risk among high-baseline BMI subjects (Supplementary Figure S8). However, none of the SNP-by-BMI interaction terms in either race were statistically significant when taking into account the race-specific multiple testing corrected thresholds for statistical significance.
Discussion

We found no statistically significant evidence of the main effects of almost 100 SNPs from seven blood pressure candidate genes on incident hypertension while taking into account baseline values of age, sex, BMI, fitness, and blood pressure (both DBP and SBP). Furthermore, the panel of SNPs tested did not modify the association between the risk of incident hypertension over 20 years and baseline fitness or BMI. The candidate gene approach of the present study has been previously used in numerous studies of hypertension with inconsistent results. A possible explanation for the lack of success of this approach may be related to an incorrect a priori assumption that predisposition to hypertension is driven by classical blood pressure regulating genes such as AGT or EDN1. As such, genome-wide association studies (GWASs) have become the most common approach to identify susceptibility genes for common diseases, since GWASs are unbiased by prior assumptions about the DNA alterations responsible for phenotypic variation. The results from recent GWASs for other complex diseases suggest that truly causative, common, consistently associated variants may not have an obvious physiological relationship to the common disease.

The evidence from GWASs for the existence of genetic susceptibility variants for hypertension remains also weak and inconsistent, and the early published GWASs for hypertension did not even find any genetic variant significantly associated with hypertension at the genome-wide level. Furthermore, none of the SNPs previously identified in candidate gene studies showed evidence of an association with hypertension in these early GWASs. These results may be caused by several factors. It is possible that hypertension may have fewer common risk alleles with large effect sizes compared to other common diseases and that the true hypertension susceptibility variants may not be represented on the chips used for GWAS genotyping. Furthermore, hypertension may be more susceptible than other complex phenotypes to misclassification bias due to the presence of latent hypertension in the control population. Phenotyping error along with population stratification, insufficient statistical power, lack of consideration of LD in the human genome, and imprecise selection of genetic markers are also possible explanations for the failure of the candidate gene approach.

A better understanding of the interactions between environmental and genetic factors constitutes a key issue in the understanding of the pathogenesis of hypertension. The evidence suggests that most of the susceptibility genes for common diseases such as hypertension do not have a strong primary etiological role in predisposition to disease, but rather act as response modifiers to exogenous environmental factors. To be sure, the context dependency of the genetic risk is evident in previous studies of hypertension. A genetic marker may have a modest effect on risk in individuals who maintain a low environmental risk, but a major effect in a high-risk environment. The ability to detect gene-environment interactions is highly dependent not only on the magnitude of effect and sample size but also on the precision with which the outcome and environmental exposures are measured. The detection of SNP-by-fitness and SNP-by-BMI interactions in the current study was aided by the objective assessment of both baseline fitness and BMI, which should help keep our potential phenotypic error low. The CARDIA study uses a
standardized data collection strategy, which supported the prospective verification of hypertension cases and controls over 20 years of follow-up. In addition, we used a strict classification of hypertension along with stringent inclusion criteria including minimizing potential racial admixture bias and controlling for baseline values of possible effect modifiers, in order to eliminate participants with prevalent disease and include only those participants that were apparently healthy and non-hypertensive at baseline.

However, there were several limitations of the present study. The number of incident hypertension cases in both races was somewhat limited which resulted in modest power to detect associations between hypertension and common SNPs, especially in white participants. Under an additive model, the genotype effect sizes detectable at 80% power on incident hypertension ranged from odds ratios (OR) of 1.51 to 1.88 in blacks and ORs from 1.70 to 2.14 in whites with MAF ranging from 0.10 to 0.50 using the multiple testing corrected thresholds (p=0.0005 in blacks and p=0.0008 in whites). These ORs are considerably higher than would be realistically expected (OR = 1.1-1.3) for common SNPs and diseases based on recent GWAS reports. For example, the number of cases that would be needed to detect OR = 1.2 with 80% power (a priori) is at least 5 times greater than in our study.

All of the nominally significant SNPs found in this study, except for the ACE rs4303 marker, were located in non-coding regions and the mechanism of their association with incident hypertension and/or interaction with fitness or BMI is unknown. Additionally, the sequence variants nominally associated with incident hypertension risk were different in blacks and whites. There are several possibilities for the observed difference. A partially different panel of markers was used in each race, as blacks and whites differed in MAF values. As exhibited in the present study, specific SNPs may have differing contributions and may not capture the same degree of information in each ethnic group. Differences in baseline values of variables such as blood pressure, BMI, and fitness may have altered the physiological pathways leading to 20 year incident hypertension in each ethnic group. Significantly fewer white participants became hypertensive over 20 years of follow-up compared to black participants. Furthermore, the association of baseline fitness and incident hypertension was not as strong in whites as it was in blacks. Since we stratified baseline fitness and BMI levels into dichotomous variables using median cut points, we may have missed potentially significant interactions that may be uncovered only at the high or low ends of the fitness and BMI distributions.

**Perspectives**

We found that the tested SNPs in selected candidate genes did not modify the association between the risk of hypertension over 20 years with baseline fitness or baseline BMI in CARDIA participants. Despite these negative results, there is a need for additional genetic association studies of hypertension that incorporate behavioral and physiological traits to better understand whether there are interactions between DNA sequence variation and lifestyle factors affecting blood pressure. However, even if such genetic and environmental interactions were shown in cohort studies, they would need to be tested in clinical trials before being used to revise treatment guidelines. Evidence from clinical trials could help
identify individuals whose blood pressure is favorably responsive to increased cardiorespiratory fitness. In ‘responsive’ individuals, support might be offered to achieve a higher level of fitness. Whereas in ‘non-responsive’ individuals, increased physical activity levels should nonetheless be promoted given the other health benefits of exercise, but other strategies for blood pressure reduction would have to be used to ensure optimal risk reduction.

**What is known about this topic**

- There is large inter-individual variation in blood pressure levels among fitness and body weight categories.
- Although numerous pathways and genes thought to be involved in blood pressure regulation have been identified, the genes that confer susceptibility to hypertension remain to be fully identified.
- While several studies have shown the association between candidate gene polymorphisms and hypertension is modified by weight status, few studies have examined whether the association between fitness level and hypertension is modified by genetic variation.

**What this study adds**

- This study examined SNP-by-fitness and SNP-by-BMI interactions on incident hypertension (over 20 years of follow-up) in a large biracial cohort.
- The SNPs from seven blood pressure candidate genes did not modify the association between fitness or BMI and risk of hypertension in CARDIA participants.
- A limitation of this study is the modest number of incident hypertension cases, which resulted in limited power to detect associations between hypertension and common SNPs, especially in white participants.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
BDKRB2 rs4900318-by-fitness interactions on the risk of incident hypertension in black (a) and white (b) participants of the CARDIA Study. Genotype distribution by hypertension and fitness group is given below the figure.
Table 1
Baseline (1985-86) characteristics, by race and year 20 (2005-06) incident hypertension status, of healthy participants without hypertension: the CARDIA Fitness Study

| Variables        | Blacks                      | Incident Hypertension Status | Whites                      | Incident Hypertension Status |
|------------------|-----------------------------|------------------------------|-----------------------------|------------------------------|
|                  | Normotensive (n=1006) mean (SD) or % | Hypertensive (n=295) mean (SD) or % | P value | Normotensive (n=1216) mean (SD) or % | Hypertensive (n=146) mean (SD) or % | P value |
| Sex (male/female) | 469/538                     | 122/173                      | 0.1134                      | 564/654                      | 86/61                        | 0.0052 |
| Age, yrs         | 23.8 (3.7)                  | 24.5 (3.7)                   | 0.0047                      | 25.2 (3.4)                   | 25.7 (3.4)                   | 0.0569 |
| Weight, kg       | 70.4 (15.6)                 | 73.8 (17.2)                  | 0.0016                      | 68.5 (13.4)                  | 72.2 (13.3)                  | 0.0013 |
| BMI, kg/m²       | 24.3 (5.0)                  | 25.7 (5.3)                   | <0.0001                     | 23.2 (3.5)                   | 24.5 (3.6)                   | <0.0001 |
| Treadmill time, min | 9.4 (2.8)                 | 8.7 (2.6)                    | 0.0002                      | 10.9 (2.6)                   | 10.6 (2.4)                   | 0.2865 |
| SBP, mmHg        | 109.1 (9.7)                 | 112.9 (9.1)                  | <0.0001                     | 107.5 (9.6)                  | 114.5 (10.1)                 | <0.0001 |
| DBP, mmHg        | 66.8 (9.0)                  | 70.3 (9.0)                   | <0.0001                     | 67.0 (8.2)                   | 71.7 (8.9)                   | <0.0001 |
| Current Smoker, % | 37.1                       | 38.1                         | 0.8721                      | 33.7                         | 35.1                         | 0.6556 |
| Education, yrs   | 13.1 (1.8)                  | 13.2 (1.9)                   | 0.3025                      | 14.7 (2.3)                   | 14.8 (2.5)                   | 0.7138 |
| Follow-up time, yrs | 20.1 (0.4)                | 20.1 (0.4)                   | 0.7782                      | 20.1 (0.4)                   | 20.2 (0.4)                   | 0.2244 |

* All variables based on baseline values except for follow-up time.
### Table 2
Multivariate odds ratios (OR) and 95% confidence intervals (CI) by race for predictors of 20 year incident hypertension: the CARDIA Fitness Study

| Variables                  | OR (95% CI)       | OR (95% CI)       |
|----------------------------|-------------------|-------------------|
|                            | Blacks            | Whites            |
| Age, yrs                   | 1.04 (1.00-1.09)  | 1.05 (0.99-1.11)  |
| Sex (M vs F) 1 vs 2        | 0.85 (0.54-1.33)  | 1.13 (0.65-1.95)  |
| BMI, kg/m²                 | 1.02 (0.98-1.05)  | 1.05 (0.99-1.11)  |
| Treadmill time, min        | 0.88* (0.81-0.95) | 0.89δ (0.80-0.98) |
| SBP, mmHg                  | 1.06† (1.03-1.08) | 1.06† (1.03-1.09) |
| DBP, mmHg                  | 1.04§ (1.02-1.06) | 1.03δ (1.01-1.06) |
| Current Smoker (Y vs N)    | 1.21 (0.88-1.67)  | 1.17 (0.79-1.74)  |
| Education, yrs             | 0.96 (0.88-1.05)  | 0.97 (0.89-1.06)  |
| Follow-up time, yrs        | 1.00 (0.69-1.45)  | 1.41 (0.89-2.20)  |

Model included all listed variables. All variables based on baseline values except for follow-up time. OR based on increase of one unit for continuous variables and 1.0 for categorical variables.

* P ≤ 0.002;
† P < 0.0001;
‡ P < 0.0002;
§ P ≤ 0.02
Table 3
Nominally significant main effects of SNPs on risk of 20 year incident hypertension, by race: the CARDIA Fitness Study

| Gene     | Chr | SNP          | Position   | p value | Minor allele homozygote OR* (95% CI) | Heterozygote OR (95% CI) |
|----------|-----|--------------|------------|---------|-------------------------------------|-------------------------|
| **Blacks, n=1301 (295 cases of incident hypertension)** |
| PPARGC1A | 4   | rs3774921    | 23,419,745 | 0.02    | 1.20 (0.79-1.81)                    | 1.55 (1.13-2.11)        |
|          | 4   | rs16874271†  | 23,488,825 | 0.03    | 1.54 (1.04-2.27)                    |                         |
| BDKRB2   | 14  | rs8016905    | 95,745,686 | 0.04    | 0.79 (0.52-1.20)                    | 0.68 (0.50-0.92)        |

| **Whites, n=1362 (146 cases of incident hypertension)** |
| Gene     | Chr | SNP          | Position   | p value | Minor allele homozygote OR* (95% CI) | Heterozygote OR (95% CI) |
|----------|-----|--------------|------------|---------|-------------------------------------|-------------------------|
| PPARGC1A | 4   | rs2932970†   | 23,427,918 | 0.048   | 1.47 (1.00-2.16)                    |                         |
|          | 4   | rs2932971†   | 23,427,982 | 0.04    | 1.47 (1.02-2.12)                    |                         |
| NOS3     | 7   | rs1808593†   | 150,339,235| 0.001   | 0.52 (0.35-0.78)                    |                         |
| BDKRB2   | 14  | rs8013400    | 95,758,361 | 0.03    | 2.43 (1.13-5.20)                    | 1.40 (0.94-2.09)        |
|          | 14  | rs4900318    | 95,758,713 | 0.004   | 2.27 (1.39-3.71)                    | 1.32 (0.86-2.03)        |
|          | 14  | rs2069571†   | 95,771,668 | 0.01    | 1.75 (1.14-2.68)                    |                         |

Chr indicates chromosome.

* The common allele homozygotes were used as the reference group for all SNPs (OR=1.0).

† For genotype groups (i.e., minor allele homozygotes with hypertension) with n <10, participants were grouped by minor allele carrier status. Thus only one OR is shown for these SNPs.
Nominally significant results for SNP-by-baseline fitness interactions on risk of incident hypertension, by race: the CARDIA Fitness Study

**Table 4**

### Blacks, n = 1301, 295 cases of incident HTN: 157 in low fitness group, 138 in high fitness group

| Gene   | Chr | SNP     | Position     | SNP main effect | SNP-by-fitness interaction |
|--------|-----|---------|--------------|-----------------|----------------------------|
| AGT    | 1   | rs2478544 | 228,910,819  | 0.96            | 0.01                       |
| NOS3   | 7   | rs3918188 | 150,333,714  | 0.47            | 0.047                      |
| BDKRB2 | 14  | rs945039  | 95,753,951   | 0.19            | 0.01                       |
|        | 14  | rs4900318 | 95,758,713   | 0.35            | 0.02                       |
|        | 14  | rs4905474 | 95,767,117   | 0.44            | 0.03                       |
| ACE    | 17  | rs4303†   | 58,911,555   | 0.31            | 0.03                       |

### Whites, n = 1362, 146 cases of incident HTN: 83 in low fitness group, 63 in high fitness group

| Gene       | Chr | SNP     | Position     | SNP main effect | SNP-by-fitness interaction |
|------------|-----|---------|--------------|-----------------|----------------------------|
| PPARGC1A   | 4   | rs2932965† | 23,414,584   | 0.03            | 0.01                       |
|            | 4   | rs6838600† | 23,484,409   | 0.09            | 0.03                       |
| EDN1       | 6   | rs2071943† | 12,403,800   | 0.34            | 0.03                       |
| BDKRB2     | 14  | rs11847625† | 95,756,049   | 0.03            | 0.006                      |
|            | 14  | rs4900318  | 95,758,713   | 0.004           | 0.005                      |

Chr indicates chromosome. SNP main effect and SNP-by-fitness interaction results are shown as p-values.

* Fitness was tested as a class variable after being dichotomized into two categories (high and low) based on race- and sex-specific median cut points for baseline treadmill time.

† Minor allele carriers of the SNP were grouped in the model(s).