Published in final edited form as:
J Hypertens. 2008 December ; 26(12): 2312–2318. doi:10.1097/HJH.0b013e3283110402.

Interactions between metallopeptidase 3 polymorphism rs679620 and BMI in predicting blood pressure in African–American women with hypertension

Jacquelyn Taylor[a], **Yan V. Sun**[b], **Jian Chu**[b], **Thomas H. Mosley**[c], and **Sharon L. Kardia**[b]
[a]Yale University, School of Nursing, New Haven, CT

[b]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI

[c]Department of Medicine, University of Mississippi Medical Center, Jackson, MS, USA

Abstract

BMI represents an internal metabolic and physiological environment that plays a key role in development of high blood pressure (BP) for many Americans. African–American women have a higher prevalence of high BP and being overweight than men or other ethnic groups. This study examines the genetic–environmental interaction effects of single nucleotide polymorphisms and BMI on BP among African–American women using 1418 African–American women and men from the Genetic Epidemiology Network of Arteriopathy study. A total of 403 tests of single nucleotide polymorphism–BMI interaction were conducted using methods of internal replication, cross-validation, and false discovery rate. One single nucleotide polymorphism (located in the *ATP6B1* gene, rs2266917) passed adjustments for multiple testing and had a significant independent main effect ($P = 0.0018$) on diastolic BP among African–American women. A significant sex-specific interaction effect was found between MMP3_rs679620 and BMI in African–American women ($P = 0.0009$). MMP3_rs679620 (A–G polymorphism) encodes a Lys-Glu nonsynonymous variant at the 45th amino acid of metallopeptidase 3 and indicates a putative functional modification of metallopeptidase 3. These findings were not identified in African–American men. MMP3_rs679620 appears to have a protective effect on diastolic BP in women with high BMI. Surprisingly, MMP3_rs679620 had the opposite effect on women with low BMI, resulting in higher diastolic BP.

Keywords

African–American; blood pressure; BMI; MMP3_rs679620; women

Introduction

Cardiovascular disease is the number one cause of death among women [1]. African–American women have the highest prevalence of cardiovascular disease (49.0%) and associated risk factors including high blood pressure (BP) (46.6%), being overweight (79.6%), and obesity (51.1%) than any other ethnic-sex group [1]. Screening for BMI based on height and weight has been shown in research to be beneficial in predicting risk for high BP [2,3]. Both environmental and genetic factors may contribute to the increased risk for

Correspondence to Jacquelyn Taylor, PhD, PNP-BC, RN, Yale University, School of Nursing, 100 Church Street South, P.O. Box 9740, Room 295, New Haven, CT 06536, USA Tel: +1 203 737 2364; fax: +1 203 785 6455; Jacquelyn.taylor@yale.edu.

high BP, therefore, exploring both these effects among African–American women is reasonable. Although genetic factors that influence individual variations in BMI have been identified, a large environmental component of weight that contributes to high BP remains to be explored. BMI represents an internal metabolic and physiological environment that plays a key role in development of high BP. Because hypertension is prevalent among African–American women and can result in severe morbidity and mortality, research on genetic susceptibility and gene–environment interactions for high BP and BMI is needed to understand and control this chronic disease. Although genetic markers have been identified as precursors for high BP, the effects that environmental factors have on the phenotypic expression of these genes must be considered. Lifestyle behaviors can prevent or enhance expression of dormant genetic predispositions. For example, consumption of foods high in fat is higher in minority and poor populations and can result in obesity and cardiovascular problems [4–6]. Healthy People 2010 identified the reduction of obesity as one of its goals for national healthcare awareness and prevention because obesity is the second highest preventable cause of death in the USA [7,8]. Maintaining an optimal weight using appropriate diet/physical activity/life style choices may impact gene expression associated with high BP. In the present study, BMI measurement testing can be used to determine effects of lifestyle behaviors on the genetic-environment interaction associated with high BP.

Methods

Study group

The Genetic Epidemiology Network of Arteriopathy (GENOA) study was initiated in 1995 to study genetics of both microvessel and macrovessel arteriopathy. In September 1996, the GENOA field centers in Jackson, Mississippi and Rochester, Minnesota began recruiting sibships that included at least two individuals diagnosed with essential hypertension before the age of 60 years. All available full biologic siblings of the index hypertensive sibling pairs (including normotensive siblings) were invited to participate in the first GENOA examination (GENOA-I) that included interviews, physical examinations, and phlebotomy. Enrollment in GENOA-I ended in June 2000, with data from interviews, physical examinations, biochemical laboratories, and clinical procedures entered into the central GENOA databases.

Measures

Height, weight, and BMI—BMI was calculated from height that was measured by stadiometer and weight by electronic balance. BMI was calculated using weight in kilograms divided by height in meters squared. BMI over 25 is considered to be overweight, and BMI outcomes greater than 30 were indicative of obesity. BMI has been shown to be a valid predictor of adiposity calculated for weight and height [9].

Blood pressure readings—BP measurements were made with random-zero sphygmomanometers and cuffs appropriate for arm size. Three readings were taken in the right arm after the participant rested in the sitting position for at least 5 min according to The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) guidelines [10]. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were determined by the first and fifth phase Korotkoff sounds, respectively, with the last two BP readings averaged for the analyses. The diagnosis of hypertension was established based on average BP levels measured at the study visit (>140/90 mmHg) or a prior diagnosis of hypertension and current treatment with antihypertensive medications. A standardized algorithm was used to define the BP diagnostic category of participants.

Genotyping—DNA was isolated using the PureGene DNA Isolation Kit from Gentra Systems (Minneapolis, Minnesota, USA). Genotyping, based on PCR amplification techniques, was conducted at the University of Texas-Health Sciences Center at Houston using the TaqMan assay and ABI Prism Sequence Detection System (Applied Biosystems, Foster City, California, USA). Primers and probes are available from the authors upon request. Quality control measures for genotyping assays included robotic liquid handling, separate pre-PCR and post-PCR areas, standard protocols, and quality control analyses including 5% duplicates, positive and negative controls, computerized sample tracking, and data validity checks. The genotyping methods have been described in detail by Barkley *et al.* [11].

Statistical methods—All analyses were carried out using the R statistical language [12]. Allele and genotype frequencies were calculated using standard gene counting methods. Linkage disequilibrium, as measured by R^2 [13] was estimated using an expectation maximization algorithm. Hardy–Weinberg equilibrium was assessed using a χ^2-test or Fisher's exact test if a genotype class had less than five individuals [14].

Associations of each of the single nucleotide polymorphism (SNP) predictors with SBP and DBP were tested using least-squares linear regression methods [15], adjusted by age, BMI and antihypertensive medication use (Yes or No). Associations involving interactions between BMI and SNPs were assessed using a partial *F*-test that compared a full model including both interaction terms and main effects with a reduced model including only main effects.

Internal replication—To create replication subsets, data from one hypertensive siblings were randomly selected from each sibship without replacement to create subset 1. Subset 2 was formed by randomly selecting another hypertensive sibling from each sibship. The GENOA cohort contained a small number of singletons (i.e., no matching siblings) who were equally divided between the two subsets.

Cross-validation—Cross-validation significantly reduced false-positive results by eliminating associations that lacked predictive ability in independent test samples. Four-fold cross-validation was performed by dividing the full sample into four equally sized groups. Three of the four groups were combined into a training dataset, and the modeling strategy outlined above was used to estimate model coefficients. These coefficients then were applied to the fourth group, the testing dataset, to predict the value of the outcome variable for each individual in the independent test sample. This process was repeated for each of the four testing sets. Predicted values for all individuals in the test set were then subtracted from their observed values, yielding the total residual variability (SSE), $\sum_{i=1}^{n}(y_i - \widehat{y_i})^2$. The total variability in the outcome (SST) (the difference between each individual's observed value and the mean value for the outcome) was then calculated, $\sum_{i=1}^{n}\left(\bar{y} - y_i\right)^2$. To estimate the proportion of variation in the outcome predicted in the independent test samples, the cross-validated R^2 (CV R^2) was calculated as follows: CV R^2 = (SST – SSE)/SST. This cross-validation provided a more accurate measure of the predictive ability of genetic models and would be negative if the model's predictive ability was poor. Because random variations in the sampling of the four mutually exclusive test groups could potentially impact estimates of CV R^2, this procedure was repeated 10 times and the CV R^2 values were averaged [16]. Univariate associations were considered cross-validated if the average percentage of variation predicted in independent test samples was greater than 0.5%. Interactions were considered cross-validated if the difference in average percentage variation predicted in

independent test samples between the full model containing the interaction term and the reduced model containing only main effect terms was greater than 0.5%.

False discovery rate—A false discovery rate adjustment was used to assess statistical significance in the face of the large number of tests. The basic false discovery rate (FDR) adjustment involved ordering the nominal *P*-values obtained for each hypothesis from the least to the greatest, with P_i being the *P*-value corresponding ith hypothesis (h_i, i 1, …, m). [17]. All h_i, $i = 1, 2, \ldots, k$, hypotheses are rejected, where k is the largest i for which $P_i = i \times$ FDR/m. The false discovery rate is defined as

$$\mathrm{FDR}=E\left[\frac{V}{R}|R>0\right]Pr\,(R>0)$$

where V is equal to the number of false-positive results and R is the number of results found significant by the statistical test. We used a resampling-based FDR procedure [18] that controlled for multiple comparisons by simulating the joint distributions of observed *P*-values under the null hypothesis of no association while considering the dependency structure of the data. Using a FDR at the 10% level as the threshold for significance, one out of every 10 significant results was considered to be a false positive.

Results

The descriptive statistics for the full sample of African–American women and men, and the two subsets used to examine replication are presented in Table 1. For the full sample, the mean age was approximately 63 years. The mean BMI for African–American women (32.7±7.0 kg/m^2) was substantially higher than African–American men (29.1±4.9 kg/m^2). The average SBP and DBP readings for African–American women were 139.4±21.7 and 78.5±10.7 mmHg, respectively. In contrast, the average SBP and DBP readings for African–American men were 136.2±20.0 and 81.5±11.0 mmHg, respectively. Eighty percent of female participants had been diagnosed with hypertension and 73% had been treated with antihypertensive medications. Among male participants, 73% had been diagnosed with hypertension and 65% had been treated with antihypertensive medications.

Table 2 presents a summary of results from testing for SNP main effects and SNP–BMI interactions, and the number of associations that remained significant after adjustment for multiple testing (FDR < 0.3), testing for replication, and cross-validation. For example, 403 SNPs were evaluated for their association with adjusted DBP and seven had FDR less than 0.3, six internally replicated, and five cross-validated. One SNP (located in the *ATP6B1* gene, rs2266917) remained significant after adjustment for multiple testing (FDR < 0.3), testing for replication, and cross-validation. The *P*-values of the two subsets and the full sample, R^2, FDR, and cross-validation R^2 were summarized in Table 3. In contrast, 403 tests of SNP–BMI interactions associated with DBP were performed, with five having a FDR less than 0.3, eight internally replicated, and five cross-validated. One SNP–BMI interaction passed all three criteria; specifically, MMP3_rs679620 interacting with BMI. The interaction between MMP3_rs679620 and waist circumstance adjusted by age and antihypertensive medication was also tested. The results indicated that the interaction effect passed all three criteria as well (Table 3). These results remained consistent even when controlling for age, antihypertensive medication, and diuretic use (Table 4). For SBP, neither the SNP main effects nor the SNP–BMI interactions passed the three test criteria: FDR, internal replication, and cross-validation. The SNP main effects and SNP–BMI interactions associated with SBP and DBP also were tested in African–American men (data not shown). None of the SNP main effects or SNP–BMI interactions passed the three test

criteria: FDR, internal replication, and cross-validation. A supplemental table has been provided for SNP annotations for the candidate genes analyzed in this study (Supplementary Table S1).

The consistent results of MMP3_rs679620 and BMI interaction effects suggest the differences in DBP levels depends on both an African–American woman's genotype and BMI (Fig. 1). In Fig. 1, we illustrate this point for SNP MMP3_rs679620 in all African–American women who were included in the sample. We found evidence that the AA and GG genotype classes were associated with higher DBP in the sample with higher BMI. However, people with the AG genotypes have lower DBP levels in the sample with higher BMI. In both subset samples, SNP MMP3_rs679620 showed the similar pattern of SNP–BMI influence on DBP (data not shown).

Discussion

A meta-analysis [19] of 21 cohort studies revealed that weight effects on BP accounts for approximately 45% of the risk for coronary heart disease. These findings remained consistent independent of other risk factors associated with high BP and coronary heart disease. Therefore, the next logical step in the literature was to examine genetic determinants of BMI and obesity. A meta-analysis [20] of 37 studies concluded that specific loci for BMI and obesity could not be found. However, the strongest linkage with possible BMI loci was associated with high BP.

Previous studies have investigated the gene–environment interaction of BMI and high BP. However, each of these studies investigated a different polymorphism or genetic region of interest, making it difficult to reach definitive conclusions about whether these associations are true or false. For example, the HyperGEN study [21] found a significant interaction between BMI and promoter polymorphisms in the angiotensinogen gene (AGT) that affects BP in whites and blacks. In comparison, the Framingham Heart Study [22] found an association between polymorphisms in the chromosome 2p region based on BMI and sex. Another study [23] claimed that the fat level in the body influences C-reactive protein and inflammation that signals polymorphisms at the catecholaminergic/β-adrenergic pathway loci thereby increasing BP. International studies have found significant associations with BMI, BP, and TGF-β1 in eastern Europeans [24] and Gln27Glu and Thr164Ile polymorphisms in predominately biracial participants in Brazil [25]. Another study based in the USA [26] found no conclusive evidence of interaction between polymorphisms in a G-protein *GNB3* and BMI influencing high BP among African–Americans.

Other researchers have concluded that although obesity has been related to increases in BP, genetic markers for obesity are not necessarily related to genetic risks for high BP among African–Americans [27]. Telgmann *et al*. [28] have determined that certain *SAH* alleles on chromosome 16 had a significant effect on BMI in white hypertensive patients, but the mechanism of the obesity related to high BP remains unknown. Although genetic studies have identified polymorphisms associated with high BP among African–Americans, little published literature bridges the gap in knowledge on BMI and how it may interact with genes to predispose African–Americans to high BP. Because a few studies have examined joint effects of genetic and environmental influences (BMI) on high BP risk among African–Americans in large epidemiological samples, this study contributes to the body of knowledge regarding the gene–environment interactions underlying high BP.

With respect to candidate gene studies, many genes and polymorphic variations have been studied, but none has predicted hypertension risk consistently. Therefore, despite some limited successes, identities and characteristics of individual genes variations that contribute

to BP levels, gene–environment interactions with BMI, and the occurrence of hypertension in the population-at-large remain poorly defined. Because African–American women have the highest prevalence of high BP and of being overweight, research on genetic susceptibility and gene–environment interactions for hypertension among these women is needed to understand and control this chronic disease better. The present study provides additional insight into the interaction of genes and the internal environment of BMI related to predicting high BP among African–American women.

After using three levels of controlling false-positive findings, internal replication, FDR, and cross-validation; a sexspecific interaction effect associated with DBP between a nonsynonymous SNP (MMP3_rs679620) of metallopeptidase 3 (MMP3), and BMI was identified. MMP3, also called stromelysin, is a member of the matrix metalloproteinase (MMP) family [29] that is involved in the breakdown of extra-cellular matrix in normal physiological processes, such as embryonic development, reproduction, tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Most MMPs are secreted as inactive proproteins that are activated when cleaved by extra-cellular proteinases. MMP3_rs679620 encodes an enzyme that degrades fibronectin, laminin, collagens, and cartilage proteoglycans. The gene is part of a cluster of MMP genes that localize to chromosome 11q22.3. Quantitative PCR provided evidence that MMP3 expression levels were negatively correlated with obesity as measured by percentage body fat [30]. The MMP3_rs679620 is also thought to be involved in progression of atherosclerosis [31].

MMP3_rs679620 (A–G polymorphism) encodes a Lys-Glu nonsynonymous variant at the 45th amino acid (in a prodomain from 18th to 99th amino acid) of MMP3. The removal of a portion of the prodomain results in conformational changes and renders them to rapid autocatalytic activation to generate fully active enzyme [32]. Although there is no direct evidence indicating the functional role of the 45th amino acid, it might be involved in the protein activation process. In its proximity [1600 base pair (bp) upstream from the MMP3 transcription start site], another MMP3 polymorphism, −11715A/6A (rs3025058), was identified, with one allele having a run of six adenosines (6A), whereas the other has five (5A) [33]. In-vitro studies of promoter strength showed that the 5A allele expressed higher activity than the 6A allele in both cultured fibro-blasts and vascular smooth muscle cells. They suggested that, because of reduced gene transcription, homozygosity for the 6A allele would be associated with lower MMP3 levels in arterial walls than other genotypes. This lower level of proteolytic activity could favor extra-cellular matrix deposition in atherosclerotic lesions.

Studies [31] have shown a relationship between MMP3, atherosclerosis, artery elasticity, and subsequent changes in SBP. Although a direct relationship between MMP3 and DBP has not been found, large artery stiffness has been associated with pulse pressure (a factor of both SBP and DBP) [31]. MMP3 modulates fragmentation of extra-cellular matrix components and decreases elastin collagen ratio, resulting in disorganized elastin networks and large arterial stiffening with age [34]. When women were compared with men, female sex steroids were found to double the fibrillin-1 deposition, influencing MMP3 variance expression that can result in large arterial stiffness [35].

Studies [36] have shown that although SBP is the best predictor of cardiovascular risk, much can be gleaned from DBP as an indicator of arterial stiffness, elevation in stroke volume, and narrowing pulse pressure. Additionally, increases in DBP have been linked to being overweight, obesity, and the metabolic syndrome, particularly among women [37]. It has been postulated that the MMP3 pathway is altered in human obesity [30]. This regulation in MMP3 activity and expression due to obesity may provide the foundation for understanding the protective effects found in this sample among participants with high BMI and low DBP.

Research on BMI has recently indicated that some additional weight in the 'overweight' category can actually be protective in preventing disease and mortality over time [38]. The authors suggest that those who are 'overweight' are protected from infection and disease due to greater nutritional reserves and higher lean body mass than people who are underweight, normal weight, or obese [38]. Therefore, the relationship between those with low-to-normal BMI with high DBP may be attributed to the arterial stiffening properties of MMP3 and lack of protective properties of having a little extra adiposity to fight off and recover from diseases such as hypertension.

The present study did not genotype the well known SNP rs3025058. However, the nonsynonymous SNP MMP3_ rs679620 was highly correlated (linkage disequilibrium R^2 = 0.98) with rs3025058 located in MMP3 promoter region among Pima Indians [30]. The significant interaction effect between MMP3_rs679620 and BMI in African–American women could also be indicative of an interaction between rs3025058 and BMI depending on the linkage disequilibrium correlation between the two SNPs in African–Americans. However, one of these SNPs was not available in HapMap and this explains why linkage disequilibrium could not be calculated. Additionally, this is why Pima Indians and MMP3 were described for further reference. These relationships have not been explored in African–Americans prior to the present study.

Some limitations of the present study need to be considered. The approach was based on the premise that susceptibility alleles for common diseases were not under strong negative selection, and common variants contributed to common disease traits (i.e. the 'common disease – common variant' hypothesis) [39]. However, the allelic spectrum for genes associated with complex quantitative traits, such as BP, was not fully delineated. It was possible that multiple rare polymorphisms in the biological and positional candidate genes that were studied could influence BP. Due to a lack of statistical power, identifying associations with BP using such alleles would not be possible using approaches employed in the present study. The inferences may not be generalizable to individuals who are younger, normotensive, or of other ethnic groups. Although a priori power calculations indicated that the study had adequate power to detect relatively small SNP effects, insufficient sample sizes (full sample and re-sampled subsets) or random measurement error may have limited the power to detect genotype–phenotype associations. The validation of the random-zero sphygmomanometers used in the GENOA study to collect BP readings was not known, but use of random-zero sphygmomanometers have been validated for many years and has been accepted for clinical investigation worldwide. Despite some limitations, the approach employed in the present study illustrated the use of SNPs in candidate genes to construct a more complete picture of the genetic architecture of complex traits, such as BP.

Genome-wide association is the currently favored approach for genetic studies of common human diseases. This approach has revealed suggestive SNP main effects associated with hypertension in a European white sample [40,41]. However, localizing and identifying genes underlying environmental variations and the occurrence of high BP poses a formidable challenge. The next step in studying African–Americans and high BP could use a similar research design incorporating a genome-wide association approach in studying gene–BMI interactions on high BP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The present work was supported by National Institute of Health grants 1 KL2 RR024987-01, P30 NR009000, 5-P30-AG015281-07, HL54457, and HL68737.

Abbreviations

CV R^2	Cross-validated R^2
FDR	False Discovery Rate
GENOA	Genetic Epidemiology Network of Arteriopathy Study
JNC-7	Evaluation, and Treatment of High Blood Pressure
MMP3	Matrix Metalloproteinase 3
PCR	Polymerase chain reaction
SNP	Single nucleotide polymorphism
SSE	Total residual variability
SST	Total variability in the outcome

References

1. Rosamond W, Flegal K, Friday G, Furie K, Go A, Greenlund K, et al. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics – 2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2007;115:e69–e171. [PubMed: 17194875]
2. Janssen I, Katzmarzyk PT, Srinivasan SR, Chen W, Malina RM, Bouchard C, Berenson GS. Combined influence of body mass index and waist circumference on coronary artery disease risk factors among children and adolescents. Pediatrics 2005;115:1623–1630. [PubMed: 15930225]
3. Moore WE, Stephens A, Wilson T, Wilson W, Eichner JE. Body mass index and blood pressure screening in a rural public school system: the Healthy Kids Project. Prev Chronic Dis 2006;3:A114. [PubMed: 16978489]
4. Boutin-Foster C, Ogedegbe G, Ravenell JE, Robbins L, Charlson ME. Ascribing meaning to hypertension: a qualitative study among African Americans with uncontrolled hypertension. Ethn Dis 2007;17:29–34. [PubMed: 17274206]
5. Douglas JG, Bakris GL, Epstein M, Ferdinand KC, Ferrario C, Flack JM, et al. Hypertension in African Americans Working Group of the International Society on Hypertension in Blacks. Management of high blood pressure in African Americans: consensus statement of the Hypertension in African Americans Working Group of the International Society on Hypertension in Blacks. Arch Intern Med 2003;163:525–541. [PubMed: 12622600]
6. Kurian AK, Cardarelli KM. Racial and ethnic differences in cardiovascular disease risk factors: a systematic review. Ethn Dis 2007;17:143–152. [PubMed: 17274224]
7. Mokdad AH, Marks JS, Stroup DF, Gerberding JL. Actual causes of death in the United States, 2000. JAMA 2004;291:1238–1245. [PubMed: 15010446]
8. US Department of Health and Human Services. Healthy People 2010. Public Health Service 2000:12.9–12.12.
9. Burton RF. Why is the body mass index calculated as mass/height(2), not as mass/height(3)? Ann Hum Biol 2007;34:656–663. [PubMed: 18092209]
10. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, National High Blood Pressure Education Program Coordinating Committee. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 2003;289:2560–2572. [PubMed: 12748199]

11. Barkley RA, Chakravarti A, Cooper RS, Ellison RC, Hunt SC, Province MA, et al. Family Blood Pressure Program. Positional identification of hypertension susceptibility genes on chromosome 2. Hypertension 2004;43:477–482. [PubMed: 14732741]
12. R Core Development Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing; Vienna, Austria: 2005.
13. Lynch, M.; Walsh, B. Genetics and analysis of quantitative traits. Sinauer Associates; Sunderland, MA: 1998.
14. Weir, B. Genetic data analysis II. Sinauer Associates; Sunderland, MA: 1996.
15. Kleinbaum, D.; Kupper, L.; Muller, K.; Nizam, A. Applied regression analysis and other multivariate methods. Brooks/Cole Publishing Company; Pacific Grove, CA: 1998.
16. Molinaro AM, Simon R, Pfeiffer RM. Prediction error estimation: a comparison of resampling methods. Bioinformatics 2005;21:3301–3307. [PubMed: 15905277]
17. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 1995;57:289–300.
18. Yekutieli D, Benjamini Y. Resampling-based false discovery rate controlling multiple test procedures for correlated test statistics. J Stat Plan Inference 1999;82:171–196.
19. Bogers RP, Bemelmans WJ, Hoogenveen RT, Boshuizen HC, Woodward M, Knekt P, et al. for the BMI-CHD Collaboration Investigators. Association of overweight with increased risk of coronary heart disease partly independent of blood pressure and cholesterol levels: a meta-analysis of 21 cohort studies including more than 300 000 persons. Arch Intern Med 2007;167:1720–1728. [PubMed: 17846390]
20. Saunders CL, Chiodini BD, Sham P, Lewis CM, Abkevich V, Adeyemo AA, et al. Meta-analysis of genome-wide linkage studies in BMI and obesity. Obesity (Silver Spring) 2007;15:2263–2275. [PubMed: 17890495]
21. Gu CC, Chang YP, Hunt SC, Schwander K, Arnett D, Djousse L, et al. Haplotype association analysis of AGT variants with hypertension-related traits: the HyperGEN study. Hum Hered 2005;60:164–176. [PubMed: 16352906]
22. Daley D, Edwards SR, Song Y, Baechle D, Puppala S, Schick JH, et al. Framingham Heart Study. Interaction of gender and body mass index (BMI) reveals evidence of linkage for hypertension in the Framingham Heart Study. BMC Genet 2003;4(Suppl 1):S45. [PubMed: 14975113]
23. Wessel J, Moratorio G, Rao F, Mahata M, Zhang L, Greene W, et al. C-reactive protein, an ‘intermediate phenotype’ for inflammation: human twin studies reveal heritability, association with blood pressure and the metabolic syndrome, and the influence of common polymorphism at catecholaminergic/beta-adrenergic pathway loci. J Hypertens 2007;25:329–343. [PubMed: 17211240]
24. Porreca E, Di Febbo C, Vitacolonna E, Baccante G, Di Castelnuovo A, Angelini A, et al. Transforming growth factor-beta1 levels in hypertensive patients: association with body mass index and leptin. Am J Hypertens 2002;15:759–765. [PubMed: 12219869]
25. Pereira AC, Floriano MS, Mota GF, Cunha RS, Herkenhoff FL, Mill JG, Krieger JE. Beta2 adrenoceptor functional gene variants, obesity, and blood pressure level interactions in the general population. Hypertension 2003;42:685–692. [PubMed: 12900437]
26. Poston WS, Haddock CK, Spertus J, Catanese DM, Pavlik VN, Hyman DJ, et al. Physical activity does not mitigate G-protein-related genetic risk for obesity in individuals of African descent. Eat Weight Disord 2002;7:68–71. [PubMed: 11930987]
27. Onions K, Hunt S, Rutkowski M, Klanke C, Su Y, Reif M, Menon A. Genetic markers at the leptin (OB) locus are not significantly linked to hypertension in African Americans. Hypertension 1998;31:1230–1234. [PubMed: 9622134]
28. Telgmann R, Brand E, Nicaud V, Hagedorn C, Beining K, Schonfelder J, et al. SAH gene variants are associated with obesity-related hypertension in Caucasians: the PEGASE Study. J Hypertens 2007;25:557–564. [PubMed: 17278971]
29. Saus J, Quinones S, Otani Y, Nagase H, Harris ED Jr, Kurkinen M. The complete primary structure of human matrix metalloproteinase-3. Identity with stromelysin. J Biol Chem 1988;263:6742–6745. [PubMed: 3360803]

30. Traurig MT, Permana PA, Nair S, Kobes S, Bogardus C, Baier LJ. Differential expression of matrix metalloproteinase 3 (MMP3) in preadipocytes/stromal vascular cells from nonobese nondiabetic versus obese nondiabetic Pima Indians. Diabetes 2006;55:3160–3165. [PubMed: 17065356]

31. Kingwell BA, Medley TL, Waddell TK, Cole TJ, Dart AM, Jennings GL. Large artery stiffness: structural and genetic aspects. Clin Exp Pharmacol Physiol 2001;28:1040–1043. [PubMed: 11903311]

32. Nagase H, Enghild JJ, Suzuki K, Salvesen G. Stepwise activation mechanisms of the precursor of matrix metalloproteinase 3 (stromelysin) by proteinases and (4-aminophenyl)mercuric acetate. Biochemistry 1990;29:5783–5789. [PubMed: 2383557]

33. Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE, Henney AM. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. J Biol Chem 1996;271:13055–13060. [PubMed: 8662692]

34. Medley TL, Kingwell BA, Gatzka CD, Pillay P, Cole TJ. Matrix metalloproteinase-3 genotype contributes to age-related aortic stiffening through modulation of gene and protein expression. Circ Res 2003;92:1254–1261. [PubMed: 12750310]

35. Natoli AK, Medley TL, Ahimastos AA, Drew BG, Thearle DJ, Dilley RJ, Kingwell BA. Sex steroids modulate human aortic smooth muscle cell matrix protein deposition and matrix metalloproteinase expression. Hypertension 2005;46:1129–1134. [PubMed: 16230520]

36. Franklin S. Arterial stiffness and diastolic blood pressure: what is the connection? Artery Res 2006;1:S1–S6.

37. Franklin S. The importance of diastolic blood pressure in predicting cardiovascular risk. J Am Soc Hypertens 2007;1:82–93. [PubMed: 20409835]

38. Flegal KM, Graubard BI, Williamson DF, Gail MH. Cause-specific excess deaths associated with underweight, overweight, and obesity. JAMA 2007;298:2028–2037. [PubMed: 17986696]

39. Reich DE, Lander ES. On the allelic spectrum of human disease. Trends Genet 2001;17:502–510. [PubMed: 11525833]

40. Levy D, Larson MG, Benjamin EJ, Newton-Cheh C, Wang TJ, Hwang SJ, et al. Framingham Heart Study 100K Project: genome-wide associations for blood pressure and arterial stiffness. BMC Med Genet 2007;8(Suppl 1):S3. [PubMed: 17903302]

41. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661–678. [PubMed: 17554300]

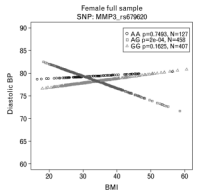

Fig. 1.

MMP3_rs679620 SNP by BMI interactions influencing diastolic blood pressure in African–American women. The relationship between the estimated values of DBP (mmHg) of all African–American women and BMI were shown for the three SNP genotypes (AA o, AG □ and GG Δ). The numbers indicate the number of subjects within each genotype group. DBP, diastolic blood pressure; SNP, single nucleotide polymorphism.

Table 1

Descriptive statistics for study participants

	Female			Male			
	Full sample (*n*=1011) (mean ± SD)	**Subset 1 (*n* = 418) (meani ± SD)**	**Subset 2 (*n* = 419) (mean ± SD)**	**Full sample (*n* = 416) (mean ± SD)**	**Subset 1 (*n* = 188) (mean ± SD)**	**Subset 2 (*n* = 188) (mean ±SD)**	***P* for sex difference**
Age (years)	62.69 (9.64)	63.16 (9.31)	63.14 (9.73)	63.63 (9.27)	63.07 (8.74)	64.76 (9.21)	0.086
BMI (kg/m^2)	32.67 (7.04)	32.75 (6.65)	32.67 (7.51)	29.13 (4.93)	29.3 (4.82)	29.07 (4.88)	<0.001
Waist circumstance (cm)	103.66 (15.19)	104.4 (15.34)	103.62 (15.54)	103.32 (12.54)	104.16 (12.93)	103.12 (11.97)	0.665
SBP (mmHg)	139.44 (21.67)	140.19 (20.98)	139.6 (22.82)	136.19 (20.02)	135.01 (18.47)	137.68 (20.87)	0.007
DBP (mmHg)	78.52 (10.72)	78.97 (10.79)	77.72 (10.95)	81.53 (11.01)	81.27 (10.28)	81.43 (11.4)	<0.001
Hypertensive, *n* (%)	808 (79.8%)	335 (80.1%)	337 (80.4%)	302 (72.6%)	135 (71.8%)	138 (73.4%)	0.008
Anti-hypertensive medication, *n* (%)	742 (73.4%)	308 (73.7%)	317 (75.7%)	269 (64.7%)	120 (63.8%)	122 (64.9%)	0.471

DBP, diastolic blood pressure; SBP, systolic blood pressure.

Table 2

Summary of the number of genetic associations with blood pressure that replicated, cross-validated, and met the false discovery rate criterion in African–American women adjusted for age and antihypertensive medication (full sample $n = 1011$, replicated samples $n_1 = 418$, $n_2 = 419$)

	Outcome: SBP		Outcome: DBP	
	SNP main effects	**SNP–BMI interaction**	**SNP main effects**	**SNP–BMI interaction**
Number of tests	403	403	403	403
$P<0.10$ on full sample	46	44	44	57
FDR (<0.30) on full sample	0	3	7	5
Cross-validation (on full sample)	0	2	5	5
Replication ($P<0.10$ in both groups)	3	12	6	8
FDR and cross-validation	0	1	5	4
FDR and replication	0	2	2	1
Replication and cross-validation	0	0	1	2
FDR and cross-validation and replication	0	0	1	1

DBP, diastolic blood pressure; FDR, false discovery rate; SBP, systolic blood pressure; SNP, single nucleotide polymorphism.

Table 3

Genetic effects that replicated, cross-validated, and passed false discovery rate criterion in female African–American (outcome: diastolic blood pressure; all associations adjusted for age and antihypertensive medications)

Main effects	SNP	Subset 1, *P*	Subset 2, *P*	Full sample, *P*	R^2	CV R^2
	ATP6B1_rs2266917	0.0929	0.0656	0.0018	0.0128	0.0051

SNP–covariate interactions	SNP	Covariate	Subset 1, *P*	Subset 2, *P*	Full sample, *P*	R^2	CV R^2
	MMP3_rs679620	BMI	0.0506	0.0848	0.0009	0.0141	0.0082
	MMP3_rs679620	Waist circumstance	0.0535	0.0849	0.0012	0.0135	0.0080

CV R^2, cross-validated R^2; SNP, single nucleotide polymorphism.

Table 4

Genetic effects that replicated, cross-validated, and passed false discovery rate criterion in female African–American (outcome: diastolic blood pressure; all associations adjusted for age, antihypertensive medications, and diuretic use)

SNP–covariate interactions	SNP	Covariate	Subset 1, *P*	Subset 2, *P*	Full sample, *P*	R^2	CV R^2
	MMP3_rs679620	BMI	0.0966	0.04892	0.0008	0.014251	0.005237
	MMP3_rs679620	Waist circumstance	0.0899	0.05025	0.000963	0.013953	0.012541

CV R^2, cross-validated R^2; SNP, single nucleotide polymorphism.