

# ORIGINAL REPORTS: HYPERTENSION

## ANALYSIS OF CANDIDATE GENES AND HYPERTENSION IN AFRICAN AMERICAN ADULTS

**Objective:** We investigated the associations between hypertension status and the genotypes of four single nucleotide polymorphism (SNP) sites in four hypertension-related genes (*Angiotensinogen* [*AGT*], *Angiotensin I Converting Enzyme* [*ACE*], *Angiotensinogen II receptor, subtype 1* [*AGTR1*], and *Alpha 1-Antichymotrypsin* [*ACT* or *SERPINA3*]), in an African American sample.

**Methods:** DNA from 628 participants of the Carolina African American Twin Study of Aging project, a population-based study of African American adult twins, was genotyped using SNPs shown to be associated with hypertension in other studies.

**Results:** The *ACE* SNP (*ACE4* or A-240T) was associated with hypertension ( $P=.047$  in a generalized estimating equations alternating logistics regression model that included age, body mass index, sex, and education. The analysis indicated a protective effect of the TT genotype (odds ratio [OR] 1.59, 95% confidence interval [CI] 1.03–2.48,  $P=.04$ ) and of the AT genotype (OR 1.91, 95% CI 1.01–3.62,  $P=.047$ ) compared with the AA genotype.

**Discussion:** These results extend previous findings of associations of various polymorphisms of *ACE* to hypertension and support the association of hypertension to the A allele of *ACE4*. The potential for this polymorphism to alter expression by its position in the gene's promoter region suggests that future studies of altered *ACE* protein activity are warranted. (*Ethn Dis.* 2009;19:18-22)

**Key Words:** African Americans, Blood Pressure, Polymorphism, Population Stratification

Keith E. Whitfield, PhD; Xiaopan Yao, PhD; K. B. Boomer, PhD;  
George P. Vogler, PhD; Mark D. Hayward, PhD;  
David J. Vandenberg, PhD

### INTRODUCTION

Hypertension is a major cause of death and functional decline among older African Americans.<sup>1,2</sup> By ages 51–61 years, more than 50% of African Americans report that they have been diagnosed with hypertension.<sup>3,4</sup> Epidemiologic studies of hypertension have documented strong associations with socioeconomic status, body mass, and smoking among both African Americans and Caucasians.<sup>3–5</sup> Progress in understanding and treating hypertension will help reduce the disparity in mortality and morbidity due to hypertension in African Americans compared with Caucasians in the United States.<sup>6</sup>

Hypertension is, in part, genetically patterned,<sup>7,8</sup> and the renin-angiotensinogen system is a biochemical pathway that regulates blood pressure that has received much attention in recent years. Three genes in particular have been associated with an increased risk for hypertension: *Angiotensinogen* (*AGT*), *Angiotensin Converting Enzyme* (*ACE*), and *Angiotensin II receptor 1* (*AGTR1*). *AGT* encodes the precursor protein that is proteolytically cleaved into angiotensin 1 by renin. *AGT* has been associated with hypertension in some<sup>9</sup> but not all studies,<sup>10–14</sup> in spite of the fact that alleles and haplotypes of the gene are consistently associated with plasma levels of *AGT*.<sup>14</sup>

Angiotensin-converting enzyme (*ACE*) catalyzes the conversion of Angiotensin 1 to the active peptide angiotensin 2, the active peptide hormone in blood pressure regulation. Several investigations have described genetic associations of multiple *ACE* polymorphisms with hypertension. Conflicting results as to a relationship between hypertension and *ACE* have been reported from linkage and association studies.<sup>15–19</sup> Most of these studies focus on an insertion/deletion polymorphism in the 16th intron, but little work has focused on the 5'-end of the gene, which displays weak linkage disequilibrium with the 3'-end of the gene.

The *AGTR1* gene encodes the angiotensin 2 receptor type 1, which regulates the hormone's cardiovascular effects, but the exact nature of a genetic association is unclear.<sup>17,20–23</sup> The same situation is present for the gene that encodes alpha 1-antichymotrypsin (*ACT* or *SERPINA3*), which is a circulating protease inhibitor of the SERPIN family, which increases in plasma in response to trauma, surgery, and infection and which acts to inhibit chymase<sup>24</sup> and cathepsin G,<sup>25</sup> both of which are able to convert angiotensin 1 to angiotensin 2. These secondary pathways in the regulation of angiotensin 2 indicate a possible indirect route by which polymorphisms in *ACT* could alter blood pressure.

In the current investigation, the main question was to what extent an association exists between hypertension status and the genotypes of single nucleotide polymorphism (SNP) sites in four relevant genes in a population-based study of African Americans. The

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From the Department of Psychology and Neuroscience, Duke University, Durham, North Carolina (KEW); Intercollege Genetics Program and Center for Developmental and Health Genetics (XY, GPV, DJV), Department of Biobehavioral Health (GPV, DJV), The Pennsylvania State University, University Park; Department of Mathematics, Bucknell University (KBB), Lewisburg, Pennsylvania; Population Research Center and Department of Sociology, University of Texas, Austin, Texas (MDH).

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Address correspondence and reprint requests to: Keith E. Whitfield; Department of Psychology and Neuroscience, Duke University; Box 90085, 9 Flowers Dr; Durham, NC 27708; kwhit1@duke.edu

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relationship between hypertension status and the four SNP sites was further investigated by examining the impact of sex, age, body mass index (BMI), and education.

## METHODS

### Participant Recruitment

This study is a part of Carolina African American Twin Study of Aging (CAATSA) project. In CAATSA, 706 persons were interviewed, which included 101 identical twin pairs, 182 fraternal twin pairs, 62 siblings, and 78 singletons.<sup>26</sup> For these analyses, singletons were excluded, which resulted in 628 participants for the analysis. Details on the registry and sample ascertainment can be found elsewhere.<sup>26</sup> Demographic information is provided in Table 1. Participants were considered hypertensive for this study even if their medications had brought their blood pressure into the normal range. Blood pressure was taken by using an oscillometric automated device (A&D Medical model UA-767, Milpitas, California). A blood pressure cuff of appropriate size was placed on participants' bare arms to record three blood pressure measurements while the participant was sitting. The assessments took place immediately after a five-minute rest period to reduce the effect of stress that may have arisen from performing the other assessments in the battery. The score for each participant was the average of the assessments.

**Table 1. Demographic characteristics of 628 participants in the Carolina African American Twin Study of Aging**

Variable	% or Mean (Standard Deviation)
Sex (male)	39
Hypertensive	40
Age, years	47.9 (13.89)
Education, years	13.3 (3.18)
Body mass index, kg/m <sup>2</sup>	29.5 (6.90)

### DNA Collection and Genotyping

Buccal DNA was collected from cotton swabs.<sup>27</sup> Some samples that were low in concentration were amplified by the whole genome amplification method by using the REPLI-g kit (Qiagen, Inc, Valencia, California).

Genotypes were generated by Taq-Man polymerase chain reaction (PCR) using the following SNP assays: *ACE*, C\_11942507\_10 (alternate names rs4291, A-240T, or *ACE4*<sup>28</sup>); *AGTR1*, C\_12080382\_10 (rs5183, A44221G); and *ACT*, C\_2188895\_10 (rs 4934, ACT codon -17A). For the *AGT* SNP (rs699, M235T), a melting curve method was used<sup>29</sup> to distinguish alleles based on differences in melting temperatures ( $T_m$ s) of PCR products after restriction digest (digested fragments have lower  $T_m$ s than the undigested parent fragment). Reactions contained forward primer 5'-AGG CTG TGA CAG GAT GGA AG-3' and reverse primer 5'-CAG GGT GCT GTC CACT GGA CCC C-3' at 2  $\mu$ mol/L, 1 $\times$  PCR buffer, 200  $\mu$ mol/L dNTP, 1.5 mmol/L MgCl<sub>2</sub>, and 2.5 U Taq enzyme in a total volume of 20  $\mu$ L. Amplification was carried out on 50 ng genomic DNA in a Gene Amp 9700 (Applied Biosystems, Foster City, California) using 35 cycles of 95°C for 60 seconds, 68°C for 60 seconds, and 72°C for 60 seconds, followed by one cycle of 9 minutes at 72°C. After PCR, the samples were digested with 1 U TthIII restriction enzyme for 2 hours. A 20- $\mu$ L aliquot of digested product was mixed with 30  $\mu$ L of 14% DASH solution, which is SYBR green 1

diluted 5000-fold in 14% formamide in water. A melting curve was generated on an ABI 7300 thermocycler. The uncut allele "T" produced a fragment of PCR-amplified DNA with a  $T_m$ =81°C and the cut allele "C" produced two fragments with a peak in the melting curve at 75°C.

### Population Structure Analysis

The method proposed by Pritchard and Rosenberg<sup>30,31</sup> was used to assess the possibility of population stratification in the sample. The test statistic was computed with STRAT software (<http://pritch.bsd.uchicago.edu/software.html>). Based on the genotype data of 90 unlinked markers, we determined whether cases and controls were appropriately matched by summing the  $\chi^2$  test statistic for case-control comparisons at each of the unlinked stratification test loci.

### Statistics

All statistical analyses were conducted in SAS version 9.1 (SAS Institute Inc, Cary, North Carolina). The difference in genotype between hypertensives and normotensives was analyzed with a generalized estimating equation alternating logistic regression (ALR) model.<sup>32,33</sup> This model separately estimates the effect of explanatory variables on hypertension status as well as the within-pair association with this outcome.<sup>34</sup> A common covariance structure was used for all sibling pair types. The model included BMI, education, sex, and a categorized age variable (> or < 60 years old).

## RESULTS

### Study Sample

In addition to the expected difference in systolic and diastolic blood pressure, hypertensives had a higher BMI (+3.1 kg/m<sup>2</sup>,  $P<.001$ ) and a lower level of education (-.64 years,  $P=.009$ ). The potential for population stratification in our sample was evaluat-

**Table 2. Genotype and allele frequencies for candidate genes and hypertension status among 628 participants in the Carolina African American Twin Study of Aging\***

Gene	Hypertension Status	Genotype % (n)			Allele % (n)	
		CC	CT	TT	%C	%T
AGT	No	22 (49)	43 (98)	35 (79)	43 (196)	57 (256)
	Yes	26 (79)	37 (114)	37 (111)	45 (272)	55 (336)
ACE	No	AA	AT	TT	%A	%T
	Yes	35 (90)	15 (38)	50 (130)	42 (218)	58 (298)
AGTR	No	AA	AG	GG	%A	%G
	Yes	48 (152)	10 (31)	42 (136)	53 (335)	47 (303)
ACT	No	AA	AG	GG	%A	%G
	Yes	52 (131)	42 (105)	6 (14)	73 (367)	27 (133)
	No	53 (165)	39 (123)	8 (25)	72 (453)	28 (173)
	Yes	53 (138)	41 (107)	6 (16)	73 (383)	27 (139)
	Yes	61 (201)	33 (108)	6 (18)	78 (510)	22 (22)

\* The number of single nucleotide polymorphisms varies slightly because of incomplete data.

ed by using the program STRAT. No significant difference was found between hypertensive subjects and control subjects ( $\chi^2=83.85$ ,  $df=90$ ,  $P=.66$ ). Thus, use of this entire sample in an analysis of hypertension should not be subject to stratification artifact.

**Genetic Analysis of Hypertension**

All markers used in this study were in Hardy-Weinberg equilibrium ( $P$  values  $>.22$ ). The frequencies of genotypes for hypertensives and normotensives are presented in Table 2. As can be seen in Table 3, *ACE* ( $P=.047$ ) showed a significant relationship with hypertensive status in the ALR model but no significant association was found for the other two SNPs ( $P=.07$  for *ACT* and  $P=.54$  for *AGTR*). Age and BMI were both highly significant in the model ( $P<.001$ ), though sex ( $P=.06$ ) and education ( $P=.23$ ) were not. Including *ACE* in the model after accounting for these factors reduced the deviance ( $P=.06$ ).

The TT and AT genotypes were less common in the hypertensive than in the normotensive participants. The odds ratios (ORs) of having hypertension between different genotypes of the *ACE* gene were significant when comparing the AA to the TT homozygote genotypes (OR 1.59, 95% confidence interval [CI] 1.03–2.48,  $P=.04$ ) and the AA to the AT genotypes (OR 1.91, 95% CI 1.01–3.62,  $P=.047$ ), which indicates that the AA genotype is a risk factor. In addition, a significant within-pair association was observed with hypertension; a person was 7.1 times more likely to have hypertension if his or her sibling had the condition than if the sibling did not ( $P<.001$ ).

**DISCUSSION**

This work presents genetic data associating a polymorphism at the 5'-end of *ACE* with hypertension, while other markers in genes of the renin

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angiotensin system displayed no associations. This result for *ACE* is of interest because much of the published genetic analyses have focused on the In/Del in intron 16 since the initial work defined this site.<sup>35</sup> Given the position of I/D polymorphism in an *Alu* repeat within this intron, this site is unlikely to be functional, but rather is probably in linkage disequilibrium with another polymorphism or polymorphisms that directly contribute to altered blood pressure. In addition, several studies have failed to demonstrate an association between the I/D polymorphism and hypertension in non-African samples, such as those of European descent.<sup>36–38</sup> The size of this gene (37 kb) and haplotype block pattern<sup>39</sup> suggest that genetic alterations in the 5'-end of the gene are in weak linkage disequilibrium with the In/Del marker and that additional genotyping to capture variation in the N-terminal region of the protein would reveal additional data on the role of *ACE* in hypertension.

The present results indicate an association of a genetic variant, *ACE4*, to variation in hypertension status in African Americans. This marker resides in an area of the gene that might serve as a promoter, and variation in other nearby sites have been associated with serum levels of *ACE*.<sup>40</sup> However, as yet, no evidence suggests a functional role for *ACE4* in controlling *ACE* promoter activity.

Genotypes of the other genes tested in the present study, *AGT*, *AGTRI*, and

**Table 3. Results of ALR analysis**

Model	Gene		Age		BMI		Sex		Education Group	
	$\chi^2$	P Value	$\chi^2$	P Value	$\chi^2$	P Value	$\chi^2$	P Value	$\chi^2$	P Value
ACE	6.10	.047	24.31	<.001	25.50	<.001	3.43	.06	1.43	.23
ACT	5.51	.07	27.69	<.001	27.13	<.001	3.33	.06	1.58	.21
AGTR	1.22	.54	29.43	<.001	25.60	<.001	3.42	.07	1.07	.30
AGT	2.80	.25	23.03	<.001	23.96	<.001	1.99	.16	1.21	.27



*ACT*, showed no association with hypertension; however, we should interpret these negative findings, and the positive finding for *ACE*, with caution. In association studies, the potential for false positive and negative results is substantial. The sample studied was population-based to diminish potential biases related to clinically recruited samples. In addition, the sample was African American, a group with high incidence of hypertension, which increases the density of genetically related cases of hypertension. We have further reduced the risk of population stratification by a specific test of 90 unlinked markers scattered across the genome. Thus, the positive association result between the *ACE* gene and hypertension status in the population of African Americans is unlikely to be due to population admixture. However, no data point to functional differences induced by this polymorphic site, and the marker used here may be in linkage disequilibrium with another causative polymorphism.

Multiple genetic factors and environmental factors are unlikely to play a role in hypertension. Two key factors highly influenced by environmental factors, BMI and age, were significant covariates. Once genes and these covariates were included in the analyses, sex was not significant. This idea is further reinforced by the fact that a significant fraction of normotensive persons had the genotype associated with hypertension in this study. A qualitative trait such as hypertension is likely to be influenced by multiple quantitative traits, each of which may be the result of multiple biochemical pathways. Thus, no one association is expected to account for a large fraction of the variance in the trait.

The present finding may not be due to the *ACE* marker genotyped but rather from the marker's being in linkage disequilibrium with a polymorphic site with functional differences that drive the phenotype. We also caution that

historically genetic associations with an OR <2 are much less likely to be replicated in subsequent studies. Future work will explore this relationship between *ACE* and hypertension further and the role of other genes and environmental effects that may affect hypertension status.

#### ACKNOWLEDGMENTS

The CAATSA was funded by a grant from the National Institute on Aging (grant #1RO1-AG13662-01A2), and the genotype analyses were supported by a grant from the Center on Population Health and Aging at The Pennsylvania State University (P30AG024395) to the first author. We also thank the CAATSA staff for assistance in phenotype data collection and data entry. Kate Anthony, Yunan Wang, and Peter Caprioli helped with purification of DNA and genotyping.

#### REFERENCES

1. Berkman L, Singer B, Manton K. Black/White differences in health status and mortality among the elderly. *Demography*. 1989;26(4):661-678.
2. Wagener DK, Molla MT, Crimmins EM, Pamuk E, Madans JH. Summary measures of population health: addressing the first goal of Healthy People 2010, improving health expectancy. *Healthy People 2010 Stat Notes*, 2001;(22):1-13.
3. Hayward MD, Crimmins EM, Milles TP, Yu Y. The significance of socioeconomic status in explaining the racial gap in chronic health conditions. *Am Sociolog Rev*. 2000;65(5):910-930.
4. Smith JP, Kington R. Demographic and economic correlates of health in old age. *Demography*. 1997;34(1):159-170.
5. Pienta A, Hayward MD, Jenkins KR. Patterns of health and marriage in later life. *J Fam Issues*. 2000;21:559-586.
6. US Department of Health and Human Services. Vital and health services: trends in the health of older Americans: United States, 1994: series 3: analytic and epidemiological studies, no. 30. In: *Services of USDHHS*, Vol Publication No 95. 1995a;1414.
7. Rotimi C, Cooper R, Ogunbiyi O, et al. Hypertension, serum angiotensinogen, and molecular variants of the angiotensinogen gene among Nigerians. *Circulation*. 1997;95(10):2348-2350.
8. Vasku A, Soucek M, Znojil V, et al. Angiotensin I-converting enzyme and angiotensinogen gene interaction and prediction of

essential hypertension. *Kidney Int*. 1998;53(6):1479-1482.

9. Jeunemaitre X, Soubrier F, Kotelevtsev YV, et al. Molecular basis of human hypertension: role of angiotensinogen. *Cell*. 1992;71(1):169-180.
10. Caulfield M, Lavender P, Farrall M, et al. Linkage of the angiotensinogen gene to essential hypertension. *N Engl J Med*. 1994;330(23):1629-1633.
11. Caulfield M, Lavender P, Newell-Price J, et al. Linkage of the angiotensinogen gene locus to human essential hypertension in African Caribbeans. *J Clin Invest*. 1995;96(2):687-692.
12. Markovic D, Tang X, Guruju M, et al. Association of angiotensinogen gene polymorphisms with essential hypertension in African Americans and Caucasians. *Hum Hered*. 2005;60(2):89-96.
13. Wu X, Luke A, Rieder M, et al. An association study of angiotensinogen polymorphisms with serum level and hypertension in an African American population. *J Hypertens*. 2003;21(10):1847-1852.
14. Fejerman L, Bouzekri N, Wu X, et al. Association between evolutionary history of angiotensinogen haplotypes and plasma levels. *Hum Genet*. 2004;115(4):310-318.
15. Zhu X, Bouzekri N, Southam L, et al. Linkage and association analysis of angiotensin I-converting enzyme (ACE)-gene polymorphisms with ACE concentration and blood pressure. *Am J Hum Genet*. 2001;68(5):1139-1148.
16. Bouzekri N, Zhu X, Jiang Y, et al. Angiotensin I-converting enzyme polymorphisms, ACE level and blood pressure among Nigerians, Jamaicans and African Americans. *Eur J Hum Genet*. 2004;12(6):460-468.
17. Henderson SO, Haiman CA, Mack W. Multiple polymorphisms in the renin-angiotensin-aldosterone system (ACE, CYP11B2, AGTR1) and their contribution to hypertension in African Americans and Latinos in the multiethnic cohort. *Am J Med Sci*. 2004;328(5):266-273.
18. Schut AF, Sayed-Tabatabaei FA, Wittman JC, et al. Smoking-dependent effects of the angiotensin-converting enzyme gene insertion/deletion polymorphism on blood pressure. *J Hypertens*. 2004;22(2):313-319.
19. Saeed Mahmood M, Saboohi K, Osman Ali S, Bokhari AM, Frossard PM. Association of the angiotensin-converting enzyme (ACE) gene G2350A dimorphism with essential hypertension. *J Hum Hypertens*. 2003;17(10):719-723.
20. Zhu X, Chang YP, Yan D, et al. Associations between hypertension and genes in the renin-angiotensin system. *Hypertension*. 2003;41(5):1027-1034.

21. Takahashi N, Murakami H, Kodama K, et al. Association of a polymorphism at the 5'-region of the angiotensin II type 1 receptor with hypertension. *Ann Hum Genet.* 2000; 64(Pt 3):197-205.
22. Barbeau P, Kulharya A, Harshfield G, Snieder H, Davis H, Treiber F. Association between angiotensin II type I receptor polymorphism and resting hemodynamics in Black and White youth. *Ethn Dis.* 2002;12(1):S1-68-71.
23. Wang X, Zhu H, Dong Y, Treiber FA, Snieder H. Effects of angiotensinogen and angiotensin II type I receptor genes on blood pressure and left ventricular mass trajectories in multiethnic youth. *Twin Res Hum Genet.* 2006;9(3): 393-402.
24. Rehault S, Brillard-Bourdet M, Juliano MA, Juliano L, Gauthier F, Moreau T. New, sensitive fluorogenic substrates for human cathepsin G based on the sequence of serpin-reactive site loops. *J Biol Chem.* 1999;274(20):13810-13817.
25. Kalsheker NA. Alpha 1-antichymotrypsin. *Int J Biochem Cell Biol.* 1996;28(9):961-964.
26. Whitfield KE, Brandon DT, Wiggins S, Vogler G, McClearn G. Does intact pair status matter in the study of African American twins? The Carolina African American Twin Study of Aging. *Exp Aging Res.* 2003;29(4): 407-423.
27. Vandenberg DJ, Anthony K, Whitfield KE. Optimizing DNA yield from buccal swabs in the elderly: attempts to promote buccal cell growth in culture. *Am J Human Biol.* 2003;15(5):637-642.
28. Villard E, Tiret L, Visvikis S, Rakotovo R, Cambien F, Soubrier F. Identification of new polymorphisms of the angiotensin I-converting enzyme (ACE) gene, and study of their relationship to plasma ACE levels by two-QTL segregation-linkage analysis. *Am J Hum Genet.* 1996;58(6):1268-1278.
29. Akey JM, Sosnoski D, Parra E, et al. Melting curve analysis of SNPs (McSNP): a gel-free and inexpensive approach for SNP genotyping. *Biotechniques.* 2001;30(2):358-362.
30. Pritchard JK, Rosenberg NA. Use of unlinked genetic markers to detect population stratification in association studies. *Am J Hum Genet.* 1999;65:220-228.
31. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics.* 2000;155: 945-959.
32. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika.* 1986;73(1):13-22.
33. Lipsitz SR, Laird NM, Harrington DP. Generalized estimating equations for correlated binary data: using the odds ratio as a measure of association. *Biometrika.* 1991;78(1):153-160.
34. Carey V, Zeger SL, Diggle P. Modeling multivariate binary data with alternating logistic regressions. *Biometrika.* 1993;80(3): 517-526.
35. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest.* 1990;86(4):1343-1346.
36. Barley J, Blackwood A, Miller M, et al. Angiotensin converting enzyme gene I/D polymorphism, blood pressure and the renin-angiotensin system in Caucasian and Afro-Caribbean peoples. *J Hum Hypertens.* 1996;10(1):31-35.
37. Schmidt S, van Hooft IM, Grobbee DE, Ganten D, Ritz E. Polymorphism of the angiotensin I converting enzyme gene is apparently not related to high blood pressure: Dutch Hypertension and Offspring Study. *J Hypertens.* 1993;11(4):345-348.
38. Vassilikoti S, Doumas M, Douma S, et al. Angiotensin converting enzyme gene polymorphism is not related to essential hypertension in a Greek population. *Am J Hypertens.* 1996;9(7):700-702.
39. Zhu X, McKenzie CA, Forrester T, et al. Localization of a small genomic region associated with elevated ACE. *Am J Hum Genet.* 2000;67(5):1144-1153.
40. Cox R, Bouzekri N, Martin S, et al. Angiotensin-1-converting enzyme (ACE) plasma concentration is influenced by multiple ACE-linked quantitative trait nucleotides. *Hum Mol Genet.* 2002;11(23): 2969-2977.

**AUTHOR CONTRIBUTIONS**

*Design concept of study:* Whitfield, Xiaopan, Vandenberg

*Acquisition of data:* Whitfield, Vandenberg

*Data analysis and interpretation:* Whitfield, Xiaopan, Boomer, Vandenberg

*Manuscript draft:* Whitfield, Xiaopan, Boomer, Vogler, Hayward, Vandenberg

*Statistical expertise:* Whitfield, Xiaopan, Boomer, Vogler, Hayward

*Acquisition of funding:* Whitfield, Hayward, Vandenberg

*Administrative, technical, or material assistance:* Whitfield, Vandenberg

*Supervision:* Whitfield, Vogler, Vandenberg