Epidemiology

Corin Gene Minor Allele Defined by 2 Missense Mutations Is Common in Blacks and Associated With High Blood Pressure and Hypertension

Daniel L. Dries, MD, MPH; Ronald G. Victor, MD; J. Eduardo Rame, MD, MPhil; Richard S. Cooper, MD; Xiaodong Wu, PhD; Xiaofeng Zhu, PhD; David Leonard, PhD; Su-Inn Ho, MA; Qingyu Wu, MD, PhD; Wendy Post, MD; Mark H. Drazner, MD, MSc

Background—The natriuretic peptide system contributes to blood pressure regulation. Atrial and brain natriuretic peptides are cleaved into smaller biologically active molecules by corin, a transmembrane serine protease expressed in cardiomyocytes.

Method and Results—This genotype-phenotype genetic association study included replication samples and genomic control to correct for population stratification. Sequencing of the human corin gene identified 2 nonsynonymous, nonconservative single nucleotide polymorphisms (Q568P and T555I) in near-complete linkage disequilibrium, thus describing a single minor I555 (P568) corin gene allele. This allele was present in the heterozygote state in ≈12% of blacks but was extremely rare in whites (<0.5% were homozygous for the minor allele). In our primary population sample, the Dallas Heart Study, after adjustment for potential confounders, including population stratification, the corin I555 (P568) allele remained independently associated with increased risk for prevalent hypertension (odds ratio, 1.63; 95% CI, 1.11 to 2.38; P=0.013). The corin I555 (P568) allele also was associated with higher systolic blood pressure in subjects not using antihypertensive medication in unadjusted (133.7±20.7 versus 129.4±17.4 mm Hg; P=0.029) and adjusted (132.5±1.6 versus 128.9±0.6 mm Hg; P=0.029) analyses. The independent association of the minor corin allele with increased risk for prevalent hypertension was confirmed in the Multi-Ethnic Study of Atherosclerosis (odds ratio, 1.50; 95% CI, 1.09 to 2.06; P=0.014). In addition, the association of the minor corin I555 (P568) allele with higher systolic blood pressure was confirmed in adjusted analysis in the Chicago Genetics of Hypertension Study (125.8±1.9 versus 121.4±0.7 mm Hg; P=0.03).

Conclusions—The corin I555 (P568) allele is common in blacks and is associated with higher blood pressure and an increased risk for prevalent hypertension. (Circulation. 2005;112:2403-2410.)

Key Words: atrial natriuretic factor ■ genes ■ genetics ■ hypertension ■ peptides

The endogenous cardiac hormonal system refers to the ability of the heart to synthesize and secrete atrial and brain natriuretic peptide (ANP and BNP) into the circulation in response to various hemodynamic stimuli, primarily myocyte stretch.1 Both ANP and BNP defend against hypertension by vasodilatation2 and natriuresis,3 as well as by opposing the actions of both the renin-angiotensin-aldosterone4 and sympathetic nervous systems.5 In mouse models, overexpression of ANP or its receptor has been shown to cause a “dose-dependent” fall in arterial blood pressure;5 selective disruption of the ANP gene (ANP−/−) or the natriuretic peptide receptor (NPRA−/−)6,8 has been shown to cause chronic murine hypertension. Whether loss-of-function mutations in the natriuretic peptide pathways contribute to some cases of human hypertension has not been determined.

Corin is a recently identified transmembrane serine protease that is highly expressed in cardiomyocytes and cleaves inactive pro-ANP and pro-BNP into smaller biologically active molecules.9-11 Here, we describe a corin allele defined by the presence of 2 nonsynonymous, nonconservative (type 1) single nucleotide polymorphisms (SNPs) in near-complete linkage disequilibrium that is enriched in blacks and associated with increased risk for prevalent hypertension and higher blood pressure.

Methods

Study Populations

Dallas Heart Study

The primary study sample was the Dallas Heart Study (DHS), a multistep probability-based sample of Dallas County residents 18 to

Received December 12, 2004; de novo received June 14, 2005; revision received July 21, 2005; accepted July 25, 2005.

From the Donald W. Reynolds Cardiovascular Clinical Research Center (D.L.D., R.V., D.L., S.-I.H., M.H.D.), Division of Cardiology (D.L.D., M.H.D.), and Hypertension Division (R.V., D.L.), University of Texas at Southwestern Medical Center, Dallas; Department of Preventive Medicine and Epidemiology, Loyola University Stritch School of Medicine, Maywood, Ill (R.S.C., X.W., X.Z.); Cardiovascular Branch, National Heart, Lung and Blood Institute, Bethesda, Md (J.E.R.); and Division of Cardiology (J.E.R., W.P.) and Donald W. Reynolds Cardiovascular Clinical Research Center (W.P.), Johns Hopkins Hospital, Baltimore, Md; and Department of Cardiovascular Research, Berlex Biosciences, Richmond, Calif (Q.W.).

Correspondence to Daniel L. Dries, MD, MPH, Heart Failure/Transplant Group, Hospital of the University of Pennsylvania, 6 Penn Tower, 3400 Spruce St, Philadelphia, PA 19104. E-mail daniel.dries@uphs.upenn.edu

© 2005 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org

DOI: 10.1161/CIRCULATIONAHA.105.568881

2403
65 years of age. The rationale, design, and sample validation of the DHS have been published. Briefly, race/ethnicity was self-assigned by the participants from the structured list of categories used in the Third National Health and Nutrition Examination Survey. Blacks were oversampled to ensure a final representation of 50%. Data were collected in 3 sequential visits: Visit 1 (n=6101) was a household interview conducted by trained field interviewers; visit 2 (n=3398) was a home visit in which blood and urine specimens were obtained; and visit 3 (n=2971) was a visit at the University of Texas Southwestern Medical Center during which imaging tests were obtained. We limited our analysis to persons in the DHS who were ≥35 years of age to increase the positive predictive value of the diagnosis of prevalent hypertension. This restricted group included most (76%) of the DHS black participants.

Multi-Ethnic Study of Atherosclerosis
The Multi-Ethnic Study of Atherosclerosis (MESA) was initiated in July 2000 to investigate the prevalence, correlates, and progression of subclinical cardiovascular disease in a population-based sample of 6814 men and women 45 to 84 years of age. Race/ethnicity was self-assigned. Details of the data collection and quality control have previously been published. Unlike the DHS and MESA, participants with known cardiovascular disease (eg, previous history of myocardial infarction or heart failure) were excluded. However, a prior diagnosis of hypertension or treatment for hypertension was not an exclusion criterion.

Chicago Genetics of Hypertension Study
The Chicago Genetics of Hypertension Study is a population-based sample of black subjects from metropolitan Chicago that comprised one of the cohorts in the International Collaborative Study on Hypertension in Blacks. Race was ascertained by self-report. Unlike the DHS and MESA, a self-reported history of hypertension or treatment for hypertension was not an exclusion criterion. Participants previously been published. Unlike the DHS, participants with known cardiovascular disease (eg, previous history of myocardial infarction or heart failure) were excluded. However, a prior diagnosis of hypertension or treatment for hypertension was not an exclusion criterion.

Blood Pressure Measurement
Dallas Heart Study
A total of 5 independent blood pressure measurements were taken in each individual at the seated position at each of the 2 in-home visits by trained and certified personnel using an automated oscillometric device ( Welch Allyn, MC ) that has been validated against direct arterial pressure measurements. A total of 5 serial measurements were made with the automated device. The average of the last 3 of the measurements was considered the blood pressure for that visit. For the present analysis, we averaged the blood pressure from the 2 in-home visits. The group means (and blood pressure distributions) were very similar between the interview sample and the phlebotomy subsamples. The group means (and blood pressure distributions) were made with the automated device. The average of the last 3 of the bivariate extreme quartiles for body mass index and 24-hour potassium. The final sample consisted of ~200 subjects in each of the bivariate extreme quartiles for body mass index and 24-hour urine sodium excretion.

Multi-Ethnic Study of Atherosclerosis
Formally trained and certified field personnel made blood pressure measurements, and data quality was centrally reviewed by the MESA Data Coordinating Center. Resting blood pressure was measured 3 times in subjects in the seated position during a single visit with a Dinamap model Pro 100 automated oscillometric sphygmomanometer (Critikon). The last 2 measurements were averaged.

Chicago Genetics of Hypertension Study
Resting blood pressure was measured at a single home visit in subjects in the seated position with an automated Omron cuff device by certified and trained field personnel. A total of 4 measurements were made in each patient in the seated position after resting at least 10 minutes. The recorded blood pressure for each participant was the average of the last 3 measurements made with the automated device. Precision and reproducibility of this automated device have been described.

Other Data Collection in the DHS
Coronary Calcium
Two consecutive electron-beam CT scans were performed on each study participant. In the present analysis, electron-beam CT scores were classified as coronary artery calcium positive when the mean electron-beam CT score was ≥10 Agatston units or as coronary artery calcium negative when the mean electron-beam CT score was ≤10 Agatston units as previously reported.

Estimated Glomerular Filtration Rate
Glomerular filtration rate was estimated by the Cockcroft-Gault equation as follows: glomerular filtration rate = (1.24×body weight in kg)/(serum creatinine in mg/dL). This value was multiplied by 0.85 to estimate the glomerular filtration rate in women.

Clinical Definitions
Hypertension
In both the DHS and MESA, hypertension was defined according to the Joint National Committee criteria as the presence of 1 of the following: (1) self-reported history of hypertension and current use of any antihypertensive medication and (2) systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg.

Diabetes Mellitus
In both the DHS and MESA, diabetes mellitus was defined by 1997 American Diabetes Association criteria as the presence of 1 of the following: (1) a fasting glucose ≥126 mg/dL or (2) a self-reported history of diabetes mellitus and current use of either insulin or an oral diabetes medication.

Sequencing and Genotyping
The corin gene was sequenced in 30 blacks by the UT Southwestern Sequencing Core Laboratory as a component of the Program in Genomic Applications. We focused on exons, the intron/exon boundaries (~30 to 50 nucleotides into the intronic regions), and the 3’- and 5’-flanking regions. The full sequencing results of the corin gene, including all SNPs identified, are available at pga@utsouthwestern.edu. We applied several criteria to prioritize identified SNPs, including a change in amino acid (nonsynonymous), especially those that were biochemically significant (nonconservative) in residues demonstrating conservation among species. Allelic discrimination of the corin variants was accomplished with 5’-nuclease assay (Taqman) and an Applied Biosystems Prism 7900 apparatus. Successful genotype determination was made in ≥99% of subjects in each of the DHS, MESA, and Chicago Genetics of Hypertension Study. Genotyping of markers that are highly informative for ancestry was performed with Perlegen Sciences high-density oligonucleotide-hybridization array technology (Perlegen Sciences). Genotyping of the DHS black population for these markers demonstrated >96% genotyping success for the 2114 markers.

Statistical Analysis
Statistical analyses were conducted with SAS 9.0 and SAS Genetics 9.0 software. Statistical comparisons of means used the Student t test, assuming unequal variances between the groups when statistically appropriate. Comparisons of proportions between groups used Pearson’s χ2 statistic. A 2-tailed probability value was used for all analyses, and a 2-sided probability value of ≤0.05 was considered statistically significant unless otherwise indicated. In multivariate linear and logistic regression analyses in the Dallas Heart Study, we adjusted for the following covariates: Age (continuous variable), gender, body mass index (continuous variable), diabetes, estimated glomerular filtration rate (continuous variable), coronary artery calcium (dichotomous variable), alcohol use, smoking status, and...
estimated African ancestry (see below). Some of these data were unavailable in our replication cohorts, precluding our ability to adjust for the exact same covariates as in the Dallas Heart Study. In the Multi-Ethnic Study of Atherosclerosis, we were able to adjust for age (continuous variable), body mass index (continuous variable), gender, and diabetes mellitus. In the Chicago Genetics of Hypertension Study, multivariate analysis included adjustment for age (continuous variable), gender, and body mass index (continuous variable). In the Dallas Heart Study and the Chicago Genetics of Hypertension Study, adjusted mean blood pressure parameters were compared between European Americans in the DHS and identified 110 sets of adjacent SNPs in strong linkage disequilibrium (see below). This final panel of 2114 SNPs was selected from an initial panel of 2270 SNPs according to the following process: Because European Americans can be considered one of the parental populations for African Americans, we first calculated the pairwise \( r^2 \) values in every chromosome in the European Americans in the DHS and identified 110 sets of adjacent SNPs in strong linkage disequilibrium (\( r^2 > 0.9 \)). Strong linkage disequilibrium between adjacent markers can seriously bias the estimation of marker location–specific ancestry; therefore, we retained only 1 SNP from each of these pairs in the subsequent analyses. SNPs that departed significantly from Hardy-Weinberg equilibrium are likely to have been inaccurately tested marker locus. It follows a \( \chi^2 \) distribution under the null hypothesis.

Results

Identification and Prevalence of Corin Gene Sequence Variants

Sequencing of the human corin gene identified 2 nonsynonymous, nonconservative (type I) SNPs in highly conserved amino acids in exon 12 (Figure 1), corresponding to a cysteine-rich frizzled-like domain. This domain has been identified as critical to the function of the catalytic domain of the protein. We genotyped the black participants in the DHS for 2114 SNPs determined to be highly informative of ancestry (see Sequencing and Genotyping above). This final panel of 2114 SNPs was selected from an initial panel of 2270 SNPs according to the following process: Because European Americans can be considered one of the parental populations for African Americans, we first calculated the pairwise \( r^2 \) values in every chromosome in the European Americans in the DHS and identified 110 sets of adjacent SNPs in strong linkage disequilibrium (\( r^2 > 0.9 \)). Strong linkage disequilibrium between adjacent markers can seriously bias the estimation of marker location–specific ancestry; therefore, we retained only 1 SNP from each of these pairs in the subsequent analyses. SNPs that departed significantly from Hardy-Weinberg equilibrium are likely to have been inaccurately genotyped, and those associated with values of \( P < 0.001 \) were also excluded. The remaining SNPs were examined with a hidden Markov model to estimate the marker allele frequencies in the parental populations. If the estimated allele frequencies at adjacent SNPs in 1 ancestral population are equal to 1.0 and 0.0 but in the other ancestral population are equal to 0.0 and 1.0, it indicates strong linkage disequilibrium between these SNPs, and only 1 of these SNPs was kept for admixture mapping. After these data verification procedures, we defined a final analytic set of 2114 SNPs to use in our statistical methods to adjust for hidden population stratification.

We estimated the marker location–specific ancestry using the hidden Markov model in which the transmission probability was calibrated from the continuous gene flow model. This method directly maximizes the likelihood function through an Expectation and Maximization iterative algorithm and allows for the uncertainty of marker allele frequencies in the parental populations (Xiaofeng Zhu, PhD, personal communication, 2005). The ancestry for each individual was estimated with the average ancestry at each marker location across the whole set of SNPs. To adjust for the population stratification, we included this estimated ancestry as a covariate in our linear regression and logistic regression models.

In addition, we applied the genomic control method by Devlin and Roeder\(^{31}\) to adjust for population stratification in association analysis for hypertension status. In this method, an inflation factor (\( \lambda \)) is first estimated using the ancestry marker panel (genomic control markers). We estimated \( \lambda = \text{median}(Y_i^2)/0.456 \), as recommended by Bacanu and colleagues,\(^{32,33}\) where \( Y_i^2, i = 1 \ldots n \), is the \( \chi^2 \) statistic for marker \( i \). Then, an adjusted test statistic \( Y^2/\lambda \) is calculated for the tested marker locus. It follows a \( \chi^2 \) distribution under the null hypothesis.

![Corin domain structure](image_url)

**Figure 1.** Corin domain structure. Corin is a type II transmembrane serine protease and, as demonstrated for other members of this family, has multiple domains between the transmembrane domain and the serine protease catalytic domain. The Q568P and T555I SNPs are located in the second cysteine-rich frizzled-like domain.
describes in reality a single corin gene minor allele defined by the presence of both SNPs on the same parental chromosome. We refer to this as the minor corin I555 (P568) allele. To reduce genotyping costs and to conserve DNA, we genotyped the remainder of the MESA subjects and the Chicago Genetics of Hypertension sample for the Q568P SNP only. Among blacks in the 3 cohorts (DHS, MESA, and Chicago Genetics of Hypertension), genotyping demonstrated that 10% to 13% of the blacks were heterozygous and 0.5% were homozygous for the corin T555 (P568) minor allele (Table 1).

In each of the cohorts, the I555 (P568) allele was in Hardy-Weinberg equilibrium.

### Baseline Characteristics Stratified by Corin I555 (P568) Allele

Given the few black homozygotes for the minor corin allele, we grouped them with the heterozygous subjects (corin variant group) and compared them with the remainder of blacks carrying no copy of the minor allele (nonvariant group). The baseline characteristics stratified by corin geno-

### TABLE 1. Prevalence of Corin Genotypes in Study Cohorts

<table>
<thead>
<tr>
<th>Corin genotype</th>
<th>DHS self-reported white participants</th>
<th>DHS self-reported black participants</th>
<th>DHS self-reported Hispanic participants</th>
<th>MESA self-reported white participants</th>
<th>MESA self-reported black participants</th>
<th>MESA self-reported Hispanic participants</th>
<th>Chicago Genetics of Hypertension Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corin $^{++}$, n (%)</td>
<td>860 (99.8)</td>
<td>1258 (86.7)</td>
<td>574 (99.1)</td>
<td>2475 (99.9)</td>
<td>1531 (88.0)</td>
<td>1406 (97.8)</td>
<td>695 (89.1)</td>
</tr>
<tr>
<td>Corin $^{+-}$, n (%)</td>
<td>2 (0.2)</td>
<td>181 (12.5)</td>
<td>5 (0.9)</td>
<td>2 (0.1)</td>
<td>199 (11.4)</td>
<td>31 (2.1)</td>
<td>81 (10.4)</td>
</tr>
<tr>
<td>Corin $^{-+}$, n (%)</td>
<td>0 (0)</td>
<td>6 (0.4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>9 (0.6)</td>
<td>1 (0.1)</td>
<td>4 (0.5)</td>
</tr>
</tbody>
</table>

Corin $^{++}$ indicates no presence of I555(P568) allele; Corin $^{+-}$, carries 1 copy of the I555 (P568) allele; and Corin $^{-+}$, carries 2 copies of the I555 (P568) allele. The I555 and P568 alleles are in near complete linkage disequilibrium.

### TABLE 2. Baseline Characteristics According to Corin Genotype

<table>
<thead>
<tr>
<th>Variant Group (Corin $^{+-}$ or Corin $^{-+}$)</th>
<th>Nonvariant Group (Corin $^{++}$)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHS (N=187)</td>
<td>(N=1258)</td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD</td>
<td>48.4 ± 8.4</td>
<td>47.6 ± 8.2</td>
</tr>
<tr>
<td>BMI, mean ± SD, kg/m²</td>
<td>30.7 ± 7.4</td>
<td>30.6 ± 7.5</td>
</tr>
<tr>
<td>Estimated GFR, mean ± SD, mL/min</td>
<td>113.4 ± 34.4</td>
<td>114.3 ± 40.0</td>
</tr>
<tr>
<td>Men, %</td>
<td>41.2</td>
<td>42.9</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>12.8</td>
<td>16.0</td>
</tr>
<tr>
<td>Current alcohol use, %</td>
<td>64.2</td>
<td>60.9</td>
</tr>
<tr>
<td>Current tobacco use, %</td>
<td>38.0</td>
<td>35.5</td>
</tr>
<tr>
<td>Current use of BP medication</td>
<td>32.6</td>
<td>30.4</td>
</tr>
<tr>
<td>CAC positive*</td>
<td>18.7</td>
<td>21.7</td>
</tr>
<tr>
<td>Self-reported history of MI</td>
<td>5.9</td>
<td>5.1</td>
</tr>
<tr>
<td>Estimated African ancestry †</td>
<td>84.4 (6.6)</td>
<td>82.6 (9.3)</td>
</tr>
<tr>
<td>MESA (N=208)</td>
<td>(N=1531)</td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD</td>
<td>62.0 ± 9.7</td>
<td>62.8 ± 10.2</td>
</tr>
<tr>
<td>BMI, mean ± SD, kg/m²</td>
<td>30.8 ± 6.5</td>
<td>30.2 ± 5.8</td>
</tr>
<tr>
<td>Men, %</td>
<td>42.3</td>
<td>45.7</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>17.3</td>
<td>15.7</td>
</tr>
<tr>
<td>Current use of BP medication, %</td>
<td>57.2</td>
<td>46.8</td>
</tr>
<tr>
<td>Chicago Genetics of Hypertension Study (N=58)</td>
<td>(N=695)</td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD</td>
<td>42.7 ± 6.1</td>
<td>42.2 ± 7.5</td>
</tr>
<tr>
<td>BMI, mean ± SD, kg/m²</td>
<td>26.6 ± 7.2</td>
<td>26.5 ± 7.5</td>
</tr>
<tr>
<td>Men, %</td>
<td>40.0</td>
<td>36.8</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; GFR, glomerular filtration rate; BP, blood pressure; and CAC, coronary artery calcium.

*Mean electron-beam CT score >10 Agatston units.
†We estimated African ancestry from 2114 highly informative markers analyzed according to the hidden Markov model, in which the transmission probability was calculated from the continuous gene flow model. See Methods for details.
type in the 3 cohorts are shown (Table 2). In the DHS, there were no significant differences between the corin variant and nonvariant groups with regard to demographic and clinical baseline characteristics. However, the mean estimated African ancestry measure (Table 2), derived from the 2114 informative markers (see Methods), was slightly but significantly higher in the corin variant group. Less detailed baseline information was available in MESA and Chicago Genetics of Hypertension, but there were no significant differences between the corin variant and nonvariant groups with regard to age, gender, and body mass index in our 2 validation cohorts or prevalent diabetes in MESA.

Figure 2. Association of the corin T555I/Q568P allele with prevalent hypertension in DHS and MESA. In DHS, the prevalence of hypertension in blacks was significantly greater in the corin variant compared with nonvariant group. Similar findings were replicated in MESA.

Association of Minor Corin Allele With Prevalent Hypertension and Blood Pressure in the DHS

As demonstrated in Figure 2, the prevalence of hypertension was significantly greater in the corin variant compared with the nonvariant group (54.5% versus 46.2%; \( P = 0.03 \)). We applied the genomic control method\(^1\) to account for potential confounding from population stratification, and the association of the corin variant group with higher prevalent hypertension remained statistically significant (\( P = 0.03 \)). In multivariate logistic regression analysis that included adjustment for differences in population admixture (Table 3), the corin variant group remained at significantly higher risk for prevalent hypertension (odds ratio [OR], 1.68; 95% CI, 1.14 to 2.48; \( P = 0.009 \)). When we analyzed the entire black cohort, including the subjects <35 years of age, the corin variant group demonstrated an increased risk for prevalent hypertension (OR, 1.51; 95% CI, 1.06 to 2.15; \( P = 0.022 \)) in multivariate analysis using the same covariates as above.

We then analyzed the data after excluding the 6 individuals who were homozygous for the minor corin allele (3 of 6, 50%, had hypertension). Multivariate logistic regression analysis that included adjustment for population admixture demonstrated that the corin variant group (all heterozygous individuals for the minor corin allele) remained independently associated with a higher risk for prevalent hypertension (OR, 1.68; 95% CI, 1.14 to 2.48; \( P = 0.009 \)). When we analyzed the entire black cohort, including the subjects <35 years of age, the corin variant group demonstrated an increased risk for prevalent hypertension (OR, 1.51; 95% CI, 1.06 to 2.15; \( P = 0.022 \)) in multivariate analysis using the same covariates as above.

We next analyzed the association of the corin T555I/Q568P allele with continuous measures of systolic, diastolic, and mean arterial blood pressures in untreated black DHS participants. We defined “untreated” as the black participants with no self-reported use of any type of antihypertensive medication based on the visit 1 survey. As demonstrated in Table 4, the corin variant group compared with the nonvariant group had significantly higher systolic and mean arterial blood pressures and a strong trend for a higher mean diastolic blood pressure in unadjusted analysis. In analysis that included an adjustment for differences in estimated population admixture and other potential confounders, the corin variant group had higher systolic, mean arterial, and diastolic blood pressures compared with the nonvariant group.

Association of Minor Corin Allele With Prevalent Hypertension and Blood Pressure in the MESA

We attempted to validate these associations in MESA. MESA was made up of an older population of blacks compared with DHS (Table 2), and the overall prevalence of hypertension was greater. As in DHS, the corin variant group had a higher prevalence of hypertension compared with the nonvariant group (Figure 2). In multivariate logistic regression (Table 5) analysis that included adjustment for age, gender, body mass

**TABLE 3. Multivariate Logistic Regression Analysis: Association of Corin Variant With Prevalent Hypertension in the DHS**

<table>
<thead>
<tr>
<th>Covariate</th>
<th>OR</th>
<th>95% CI</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corin (variant vs nonvariant group)*</td>
<td>1.63</td>
<td>1.11–2.38</td>
<td>0.013</td>
</tr>
<tr>
<td>Age, per 1-y increase</td>
<td>1.09</td>
<td>1.07–1.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender, men vs women</td>
<td>0.99</td>
<td>0.75–1.31</td>
<td>0.96</td>
</tr>
<tr>
<td>BMI</td>
<td>1.09</td>
<td>1.06–1.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes, yes vs no</td>
<td>2.13</td>
<td>1.44–3.16</td>
<td>0.0002</td>
</tr>
<tr>
<td>GFR, per 1-mL/min increase</td>
<td>0.98</td>
<td>0.97–0.99</td>
<td>0.02</td>
</tr>
<tr>
<td>CAC positive (yes/no)†</td>
<td>1.55</td>
<td>1.12–2.16</td>
<td>0.009</td>
</tr>
<tr>
<td>Alcohol use (yes/no)</td>
<td>0.96</td>
<td>0.73–1.28</td>
<td>0.79</td>
</tr>
<tr>
<td>Current smoker (yes/no)</td>
<td>1.44</td>
<td>1.08–1.93</td>
<td>0.01</td>
</tr>
<tr>
<td>Estimated African ancestry, per 1% increase‡</td>
<td>1.01</td>
<td>0.99–1.03</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 2.

*Corin variant group includes heterozygotes and homozygous recessives for minor corin T555I (Q568P) allele.
†Mean electron-beam CT score >10 Agatston units.
‡To control population stratification, 2 statistical methods were applied to our original analyses for association based on a total of 2114 SNPs that are highly informative for ancestry. The marker location–specific ancestry was estimated with the hidden Markov model, in which the transmission probability was calculated from the continuous gene flow that directly maximizes the likelihood function through an EM iterative algorithm and allows the uncertainty of marker allele frequencies in the parental populations. The ancestry for each individual was estimated with the average ancestry at each marker location across the whole set of SNPs derived from 2114 highly informative SNPs (see Methods for details).
TABLE 4. DHS: Comparing Unadjusted and Adjusted Mean Blood Pressure Parameters in Corin Genotype Groups Among Untreated* Black Subjects

<table>
<thead>
<tr>
<th>BP Parameter</th>
<th>Corin+/– (n=123)</th>
<th>Corin−/− (n=3)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted mean±SD, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>133.7±20.7</td>
<td>129.4±17.4</td>
<td>0.027</td>
</tr>
<tr>
<td>MAP</td>
<td>101.8±13.2</td>
<td>97.6±11.4</td>
<td>0.042</td>
</tr>
<tr>
<td>DBP</td>
<td>84.3±10.4</td>
<td>81.2±9.1</td>
<td>0.066</td>
</tr>
<tr>
<td>Adjusted† mean±SEM, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>132.5±1.6</td>
<td>128.9±0.6</td>
<td>0.029</td>
</tr>
<tr>
<td>MAP</td>
<td>99.3±1.1</td>
<td>96.4±0.4</td>
<td>0.013</td>
</tr>
<tr>
<td>DBP</td>
<td>82.7±0.9</td>
<td>80.2±0.3</td>
<td>0.010</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; MAP, mean arterial blood pressure; DBP, diastolic blood pressure; Corin+/–, no presence of the I555(P568) allele; Corin−/–, carries 1 copy of the I555 (P568) allele; and Corin−/−, carries 2 copies of the I555 (P568) allele.

*Untreated defined as no self-reported use of any type of antihypertensive medication at the time of in-house visit 1.
†Adjusted least-squares estimate of blood pressure parameter. It is adjusted for the following covariates: Age, gender, body mass index, prevalent diabetes mellitus, coronary calcium (yes/no), self-reported alcohol consumption (current drinker/non drinker), self-reported smoking status (current smoker/non smoker), estimated glomerular filtration rate, and ancestry variable (continuous variable) to correct for population stratification.

index, and prevalent diabetes, the presence of the I555 (P568) allele remained independently associated with a higher risk for hypertension in MESA. However, unlike the DHS, when we analyzed the untreated black participants, there were no significant differences in the mean systolic, diastolic, or mean arterial blood pressures in the corin variant and nonvariant groups. Of note, more subjects in the corin variant group (114 of 208, 55%) than in the nonvariant group (728 of 1531, 48%) were being treated with antihypertensive medication at the time of enrollment into cohort as defined according to entry criteria.

Assessment of Dose-Response Effect of Minor Corin Allele

We combined the black participants in the DHS and MESA to examine a genotype-phenotype “dose-response” relationship given the small number of homozygous participants in each of the 2 individual cohorts. There was the suggestion of an increase in the prevalence of hypertension across the genotype groups in the combined cohort: participants homozygous for major allele (1454 of 2789, 52%), heterozygous for minor corin allele (228 of 380, 60%), and homozygous for minor allele (10 of 15, 66%) (P=0.002 for trend). However, the small number of homozygous subjects weakens the strength of this conclusion.

Association of Minor Corin Allele With Blood Pressure in the Chicago Genetics of Hypertension Study

The Chicago Genetics of Hypertension Study provided the opportunity to validate the association of the corin 1555 (P568) allele with continuous blood pressure parameters because by design all participants were not using antihypertensive medication. The Chicago Genetics of Hypertension Study included a population with an age range (Table 2) similar to that of the black participants in the DHS. Unlike the DHS and MESA, this study excluded participants with a self-reported prior diagnosis of hypertension or the current use of an antihypertensive medication, creating the potential for bias against finding an association of the minor corin allele with higher blood pressure. Nonetheless, despite this potential, the corin variant group demonstrated significantly higher systolic and mean arterial blood pressures (Table 6) and a similar trend for higher diastolic blood pressure. These results persisted in adjusted analysis.

Discussion

The data herein describe a minor corin 1555 (P568) allele characterized by the presence of 2 nonsynonymous, nonconservative (type 1) SNPs that are enriched in blacks and associated with a higher risk for hypertension and higher blood pressure in untreated individuals. The increasing number of recognized signaling pathways and molecules involved in mammalian blood pressure regulation, small biological effects attributable to single polymorphisms, and conceptual and methodological study limitations have provided significant challenges to dissect the genetic contributions to interindividual variability in blood pressure and risk for the
development of hypertension. Lack of reproducibility of genetic association studies for complex traits has created concern for false-positive associations and prompted prominent editorial boards to recommend that investigators seek replication of positive associations in independent population samples.\textsuperscript{34}

Given these concerns, we believe that the replication of the results demonstrated in our primary cohort, the Dallas Heart Study, in 2 additional, independent population-based cohorts (MESA and Chicago Genetics of Hypertension Study) is a major strength of the present analysis. Such replication reduces the risk for a false-positive association caused by multiple testing. In addition, in our primary cohort (DHS), we were able to control for population stratification, another major cause of false-positive gene association studies, using 2 validated statistical methods employing 2114 highly informative SNPs.\textsuperscript{24} The corin variant group demonstrated a small but statistically significant greater estimate of African ancestry. However, adjusting our data for population stratification by 2 different methods did not reduce the association of the minor corin allele with a higher risk for prevalent hypertension or the association of the minor corin allele with higher blood pressure. Our approach was based on an analysis of SNPs that were prioritized on the basis of several characteristics described earlier. Such approaches require testing multiple SNPs for association and raises concerns about multiple testing. However, replication of the association to various degrees in 2 independent cohorts reduces the possibility of a type I error. Another approach that we did not use that may increase statistical power and reduce the problem of multiple testing is to incorporate a haplotype-based approach across the candidate gene region.

Corin is a plausible candidate gene for association with hypertension on the basis of the recognized importance of the natriuretic peptide system to blood pressure regulation.\textsuperscript{35–38} At the most proximate limb of the natriuretic peptide system, corin is required to process natriuretic peptide precursors effectively into smaller biologically active molecules. Impairment of this process might contribute to altered blood pressure regulation, as suggested recently from the report that homologous deletion of the corin gene in mice results in higher blood pressure and cardiac hypertrophy in the corin gene knockout mice.\textsuperscript{39} Additionally, the minor corin allele is defined by the presence of 2 missense SNPs, and the amino acid changes are nonconservative, occur in an important functional domain, and demonstrate strong conservation among species. Finally, the magnitude of the increased odds for prevalent hypertension in the corin variant group is consistent with the expected magnitude for a single gene contribution to complex phenotypes, as demonstrated by previous gene association studies for complex traits that have been successfully replicated.\textsuperscript{45}

Our 3 different cohorts provided complementary strengths to determine the association of the minor corin allele with the risk for higher blood pressure and hypertension. The DHS and Chicago Genetics of Hypertension trials recruited large numbers of younger untreated black subjects, thereby providing the opportunity to test for linear associations with blood pressure levels when analyzed as continuous variables. In contrast, MESA provided the opportunity to confirm the hypothesized association between corin genotype and prevalent hypertension in a significantly older population in which hypertension was very common and antihypertensive treatment had already been initiated in a substantial proportion of participants. However, the greater prevalence of treated hypertension in MESA likely contributed to our inability to replicate the association of the corin minor allele with blood pressure levels in untreated subjects.

We recognize that in the absence of functional studies, we cannot conclude that the association of the minor corin T555I (Q568P) allele with higher blood pressure and risk for prevalent hypertension was mediated by a reduction in the ability of corin to process pronatriuretic peptides into their biologically active moieties. Rather, T555I and Q568P may be in linkage disequilibrium with another unrecognized causal variant. It is intriguing, however, to hypothesize that T555I and/or Q568P may alter the bioactivity of corin. These polymorphisms result in nonconservative amino acid substitutions within the second cysteine-rich frizzled-like domain in exon 12 of corin. The cysteine-rich frizzled domains are important for Wnt-signaling pathways, protein-protein interactions,\textsuperscript{41,42} and adequate catalytic activity of corin. Deletion of the first cysteine-rich frizzled domain reduced the ability of corin to process pro-ANP by 40%.\textsuperscript{33} Similarly, deletion of the second cysteine-rich frizzled-like domain, in which T555I and Q568P are located, reduced the catalytic function of corin by 50% to 60% (Q.W., Berlex Biosciences, personal communication).

The present study has limitations. Using a candidate gene approach, we have tested multiple SNPs for association with hypertension, which raises concerns about an increased risk of false-positive associations. To reduce this risk, however, we were committed a priori to replication of initial associations in additional independent cohorts. We were unable to replicate the association of the corin genotype with untreated blood pressure levels in MESA, but we reason that this is explained by the small number of untreated hypertensive participants in MESA and the exclusion of persons with known cardiovascular disease, which may have biased against our phenotype of interest. The small number of subjects homozygous for the minor I555 (P568) allele limited our ability to test a genotype-phenotype dose response across the 3 genotype groups.

It should be acknowledged that despite the demonstrated higher relative odds for prevalent hypertension associated with the minor corin T555I (Q568P) allele, the attributable risk for hypertension associated with the corin T555I/Q568P allele will be modest (≈2% to 3%) and cannot explain the excess hypertension in US blacks relative to whites. The potential exists that overemphasis on genetics as a major explanatory factor for health disparities may result in researchers overlooking other equally important factors contributing to these disparities and may inadvertently reinforce racial stereotyping, as recently pointed out by others.\textsuperscript{43}

In conclusion, the present data demonstrate that the corin I555 (P568) allele was enriched in blacks and was associated with higher blood pressure and a higher risk for prevalent hypertension.

Acknowledgments

Drs Dries, Victor, Leonard, Cooper, Rame, and Drazner received support from the Donald W. Reynolds Cardiovascular Clinical
Research Center, Dallas, Tex. Drs Dries and Dzavran were supported as Reynolds Associates. In addition, Dr Dries received support from the NIH, NHLBI (K23-HL04455). Dr Dzavran was the recipient of a Doris Duke Clinical Scientist Development Award from the Doris Duke Charitable Foundation, New York, NY. Dr Cooper received support from the NIH, NHLBI (RO-1 45508; 47910). Drs Cooper and Post are investigators for the NHLBI-funded MESA. Dr Post is a Paul Beeson Physician Faculty Scholars in Aging Research Award recipient and a Reynolds Associate.

Disclosure
Dr Wu is consultant for and former employee of Berlex Biosciences.

References
Corin Gene Minor Allele Defined by 2 Missense Mutations Is Common in Blacks and Associated With High Blood Pressure and Hypertension

Daniel L. Dries, Ronald G. Victor, J. Eduardo Rame, Richard S. Cooper, Xiaodong Wu, Xiaofeng Zhu, David Leonard, Su-Inn Ho, Qingyu Wu, Wendy Post and Mark H. Drazner

Circulation. 2005;112:2403-2410; originally published online October 10, 2005; doi: 10.1161/CIRCULATIONAHA.105.568881

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/112/16/2403

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/