A Population Association Study of Angiotensinogen Polymorphisms and Haplotypes With Left Ventricular Phenotypes

Laura J. Rasmussen-Torvik, Kari E. North, C. Charles Gu, Cora E. Lewis, Jemma B. Wilk, Aravinda Chakravarti, Yen-Pei C. Chang, Michael B. Miller, Na Li, Richard B. Devereux, Donna K. Arnett

Abstract—Several studies have shown an association between single nucleotide polymorphisms (SNPs) in the angiotensinogen (AGT) gene and hypertension. Because hypertension is a risk factor for left ventricular (LV) hypertrophy and because evidence from animal models suggests that AGT may play a role in the growth and hypertrophy of the heart, we chose to conduct a population association study examining the relationship of 10 SNPs in the AGT gene with 7 different LV phenotypes measured by echocardiography. Participants (336 whites and 441 blacks) were drawn from the Hypertension Genetic Epidemiology Network (HyperGEN) study. Individuals were genotyped for 10 previously identified SNPs within the AGT gene. SNP genotype results were regressed against continuous LV phenotypes to test associations separately in each race. Using a cutoff of $P<0.005$ to account for multiple testing, we found 1 SNP (rs943580) significantly associated with transmural early peak filling velocity (MVE) in the black population. We also used Phase 2.0.2 to reconstruct haplotypes from genotype data. Using the same cutoff of $P<0.005$, we found no haplotypes to be significantly associated with the LV phenotypes. To better understand the association between rs943580 and MVE, we examined AGT haplotype associations with MVE. The single SNP association was driven by a large group of SNPs in high linkage disequilibrium that includes the promoter SNP rs5051. (Hypertension. 2005;46:1294-1299.)

Key Words: population ■ genetics ■ hypertrophy ■ remodeling ■ angiotensinogen ■ haplotypes

Left ventricular (LV) hypertrophy, defined categorically as LV mass above the 97.5th percentile of the LV mass distribution in normotensive, normal-weight individuals, is a common cardiac disorder in the United States. Studies have shown the prevalence of LV hypertrophy (LVH) to be 16% in whites and 33% to 43% in blacks.1,2 LVH is a powerful predictor of morbidity and mortality from myocardial infarction, stroke, and congestive heart failure.3,4 Given this, and the ease of measuring LV mass and function using echocardiography in large population samples, echocardiographic measures of the left ventricle can be important predictive phenotypes of cardiovascular morbidity and mortality. Studies have also shown that LV mass is a heritable trait. In the Framingham Heart Study, the heritability of LV mass was estimated to be between 0.24 and 0.32,5 and in the HyperGEN study, the sibling correlation of LV mass was between 0.29 and 0.44 in blacks.6 These moderate levels of heritability suggest there may be genetic risk factors for LVH.

Angiotensinogen (AGT) is a gene spanning 13 kb on chromosome 1q42. The role of AGT in controlling blood pressure as part of the renin-AGT-aldosterone system is well understood. Many recent studies have examined the association of several known single nucleotide polymorphisms (SNPs) in the AGT gene with the prevalence of hypertension. Results from several populations have indicated that 2 SNPs (M235T and G–6 A) are associated with increased prevalence of hypertension,7,8 although these results have not been confirmed in all populations.9 Additionally, studies have shown increased AGT synthesis in the myointimal layer after injury to an artery.10 Given this evidence and the association of hypertension with LVH, there has been recent interest in the association of AGT SNPs with measures of LV size and function. The M235T AGT SNP has been shown to be associated with LV mass in 2 cross-sectional studies,11,12 but not another,13 and to be associated with reduction in indexed LV mass among those taking an angiotensin receptor blocker but not a β-blocker in a treatment trial.14 The Cardiogenomics group found that the AGT rs7079 SNP (a...
noncoding SNP in the exon 5 UTR) is associated with LV mass in women in the Framingham Heart Study. In this study, we seek to duplicate results of earlier studies and also examine the association of as yet untested echocardiographic phenotypes with SNPs in the AGT gene.

Methods

Study Population

Individuals in this study were participants in the HyperGEN study, which is 1 of 4 networks in the National Heart, Lung, and Blood Institute Family Blood Pressure Program (FBPP). Recruitment procedures for HyperGEN have been reported previously. This analysis used SNP genotypes typed on a subset of the total HyperGEN population selected for a case-control study of hypertension. For that study, a biracial sample (blacks and whites) of 536 severe hypertensive cases and 415 independent normotensive controls together with their offspring were selected. To create an unrelated sample for this study, we first selected all participants who were blinded to participants’ clinical data. LV internal dimension and interventricular septal and posterior wall thickness were measured at end-diastole and end-systole by American Society of Echocardiography recommendations on up to 3 cardiac cycles at or just below the tips of mitral leaflets on parasternal long-axis and short-axis views. When optimal orientation of the LV M-mode could not be obtained, correctly oriented linear dimensions were measured from 2D imaging by the leading-edge American Society of Echocardiography convention. LV diastolic filling parameters were measured by pulsed Doppler in apical 4-chamber view during diastole, with the sample volume placed at mitral valve leaflet tips or mitral valve annulus. LV mass, aortic root diameter, atrial phase peak filling velocity MVA, transmirtal early peak filling velocity (MVE), mid-wall shortening (MWS), relative wall thickness (RWT), and LV internal diameter (LVDD) were calculated as described previously. LV mass was indexed by height (creating the variable LV mass/(HT2.7)) to control for the effect of body size. Interclass correlation coefficients of methods similar to those used in HyperGEN have been reported by the reading center (r = 0.94 for LV mass indexed by height, 0.58 for MVE, 0.57 for MVA, 0.68 for MWS, and 0.87 for LVDD).

More than half (56%) of subjects with echocardiogram data had filling velocity measurements at the leaflet tips, and 38% had velocity measurements at the annulus. For those missing measurements at the tips, equations derived for hypertensive patients involved in the Losartan Intervention of Endpoint (LIFE) trial were used to convert filling velocity measurements at the annulus to filling velocity measures at the tips. With these conversions, 310 whites and 416 blacks had MVA measurements, and 309 whites and 419 blacks had MVE measurements. For all phenotypes, trait values were presented as means ± SD. *P < 0.05 for a 2-sided t test comparing group means.

Genotypes

All genotyping took place in the laboratory of A.C. at Johns Hopkins University using DNA prepared from whole blood. Ten SNP markers covering the whole genomic region of AGT were selected (Figure 1; Table 2). All SNPs except for rs699 (T235 in Exon2) were genotyped using a 5′nuclease-based method (TaqMan; Applied Biosystems); rs699 was genotyped using single-base extension with labeled nucleotides followed by detection using fluorescence polarization. The AcycloPrime-FP SNP detection kit and Victor2 readers offered by Perkin–Elmer were used in the genotyping.

Phenotypes

Echocardiograms, blood pressure readings, and anthropomorphic measures were taken at each of the field centers. Echocardiograms were performed locally by centrally trained sonographers. The echocardiographic methods used and protocols followed have been described previously. Readings were made and phenotypes derived at the central reading center at the Weill Cornell Medical Center by experienced sonographers and verified by trained cardiologists, all of whom were blinded to participants’ clinical data. LV internal dimension and interventricular septal and posterior wall thickness were measured at end-diastole and end-systole by American Society of Echocardiography recommendations on up to 3 cardiac cycles at or just below the tips of mitral leaflets on parasternal long-axis and short-axis views. When optimal orientation of the LV M-mode could not be obtained, correctly oriented linear dimensions were measured from 2D imaging by the leading-edge American Society of Echocardiography convention. LV diastolic filling parameters were measured by pulsed Doppler in apical 4-chamber view during diastole, with the sample volume placed at mitral valve leaflet tips or mitral valve annulus. LV mass, aortic root diameter, atrial phase peak filling velocity MVA, transmirtal early peak filling velocity (MVE), mid-wall shortening (MWS), relative wall thickness (RWT), and LV internal diameter (LVDD) were calculated as described previously. LV mass was indexed by height (creating the variable LV mass/(HT2.7)) to control for the effect of body size. Interclass correlation coefficients of methods similar to those used in HyperGEN have been reported by the reading center (r = 0.94 for LV mass indexed by height, 0.58 for MVE, 0.57 for MVA, 0.68 for MWS, and 0.87 for LVDD).

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**TABLE 1. Characteristics of the Study Population**

<table>
<thead>
<tr>
<th>Variable</th>
<th>White (n=336)</th>
<th>Black (n=441)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>46.1</td>
<td>46.3</td>
</tr>
<tr>
<td>Hypertensive (%)</td>
<td>54.2</td>
<td>48.7</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>60.2±8.6</td>
<td>44.9±11.6</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>29.6±6.6</td>
<td>31.4±7.7</td>
</tr>
<tr>
<td>Aortic root diameter (cm)*</td>
<td>3.49±0.41</td>
<td>3.33±0.38</td>
</tr>
<tr>
<td>MVA (cm/s)</td>
<td>72.8±17.4</td>
<td>73.3±17.8</td>
</tr>
<tr>
<td>MVE (cm/s)*</td>
<td>67.4±16.4</td>
<td>81.6±18.3</td>
</tr>
<tr>
<td>MWS (%)</td>
<td>17.6±2.5</td>
<td>17.3±2.4</td>
</tr>
<tr>
<td>RWT</td>
<td>0.34±0.05</td>
<td>0.34±0.05</td>
</tr>
<tr>
<td>LVM/HT2.7</td>
<td>40.4±10.3</td>
<td>41.9±12.8</td>
</tr>
<tr>
<td>LVDD (cm)</td>
<td>5.12±0.55</td>
<td>5.18±0.53</td>
</tr>
<tr>
<td>Stroke volume (mL/beat)</td>
<td>75.7±15.9</td>
<td>76.0±15.7</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD.

*P < 0.05 for a 2-sided t test comparing group means.

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**TABLE 2. AGT SNPs**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Function</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs5046</td>
<td>−532</td>
<td>Noncoding</td>
<td>Promoter</td>
</tr>
<tr>
<td>rs5049</td>
<td>−217</td>
<td>Gluc-HRE†</td>
<td>Promoter</td>
</tr>
<tr>
<td>rs5050</td>
<td>−20</td>
<td>ERE‡</td>
<td>Promoter</td>
</tr>
<tr>
<td>rs5051</td>
<td>−6</td>
<td>Noncoding</td>
<td>Promoter</td>
</tr>
<tr>
<td>rs2493134</td>
<td>507</td>
<td>Noncoding</td>
<td>Intron 1</td>
</tr>
<tr>
<td>rs4762</td>
<td>3889</td>
<td>Thr174Met</td>
<td>Exon 2</td>
</tr>
<tr>
<td>rs699</td>
<td>4072</td>
<td>Met235Thr</td>
<td>Exon 2</td>
</tr>
<tr>
<td>rs2493132</td>
<td>6309</td>
<td>Noncoding</td>
<td>Intron 2</td>
</tr>
<tr>
<td>rs7079</td>
<td>11535</td>
<td>Noncoding</td>
<td>Exon 5 UTR</td>
</tr>
<tr>
<td>rs943580</td>
<td>12775</td>
<td>Noncoding</td>
<td>3′</td>
</tr>
</tbody>
</table>

†Glucocorticoid hormone response element; ‡estrogen response element.

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**Figure 1. Angiotensigen.**

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exceeding 2.5\times the interquartile range above or below the median trait value were discarded.

**Data Analysis**

Individual SNP associations with continuous LV phenotypes were calculated using linear regression in PC SAS version 8.0. Models were also adjusted for age and sex (and heart rate for MVA, MVE, and LVIDD variables only). Adjusted means of the LV phenotypes by SNP genotype were calculated with the "lmmeans" feature of "proc GLM" in PC SAS version 8.0. To avoid possible confounding attributable to different allele frequencies, and in recognition of the fact that different genetic variants may be at play in the black and white populations, all analyses were performed separately by race.

Because we tested 10 SNPs for each phenotype, we set a threshold of \( P<0.005 \) as a measure of statistical significance. In an additional effort to correct for multiple comparisons, we also calculated empirical \( P \) values for any single SNP associations that met our level of significance. To do this, we created 1000 data sets by randomly permuting phenotypic values on genotypic values within race-specific data sets. Adjusted associations for a given echocardiographic trait and each of the 10 SNPs were calculated for each data set, and the greatest \( P \)-test value among these 10 tests was recorded. The greatest \( F \)-tests from all 1000 data sets were then ranked and the empirical \( P \)-value determined by the position of the original \( F \)-test fell on the ranked list.

Phase v2.0.2 (a program that uses a Bayesian statistical method to reconstruct haplotypes from population genotype data\cite{26,27}) was used to infer haplotypes from all 10 SNP genotypes for the unrelated white and black subpopulations separately. The predicted most likely haplotypes were then used to compute race-specific estimates of \( r^2 \) for all 10 SNPs (see Li section 1.3 for equations\cite{28}) and used for regression analyses. Using the "Proc GLM" procedure in PC SAS version 8.0, haplotype associations and adjusted means of all LV phenotypes were calculated. Haplotypes having a \( \geq 5\% \) total frequency in the population ("major haplotypes") were included individually in models as 2-\( df \) variables (with the groups consisting of individuals with 2, 1, or 0 copies of the haplotype in question) and also included in 1-\( df \) models (where all those having 1 or 2 copies of the haplotype were grouped together). Again, because we tested the association of multiple haplotypes with each LV phenotype, we set a threshold of \( P<0.005 \) as a measure of significance. All haplotype regressions were adjusted for age, sex, hypertension status, and heart rate, and haplotype regressions were weighted by the posterior probabilities of the predicted haplotype pairs, as calculated by the Phase program. Post hoc power analyses were conducted with the program G*Power.

**Results**

Table 1 lists the characteristics of the black and white study populations. Because the initial study population was selected on hypertension, both samples had roughly equal numbers of normotensive and hypertensive individuals. The table indicates phenotypes that differed significantly between the races.

The results of the single SNP association analysis are presented in Figure 2. No association achieved statistical significance using the \( P<0.005 \) threshold in the white population. Only 1 association (rs943580 and MVE) achieved our preset level of significance in the black population. When an empirical \( P \) value for this association was calculated, the actual \( F \)-test value for the original association fell 981st on the list of \( F \)-test values calculated from permuted data sets, resulting in an empirical \( P \) value of 0.019.

The correlations (\( r^2 \)) among the 10 typed SNPs are listed for each race in Table 3. Comparing the calculations for whites and blacks reveals different patterns of linkage disequilibrium between the 2 races. Further examining the pattern of linkage disequilibrium in the black population reveals that 3 SNPs are highly correlated (\( r^2>0.7 \)) with the rs943580 SNP.

Table 4 lists the adjusted means of MVE by rs699 and rs5051 (2 highly correlated SNPs associated with hypertension in other analyses) are presented. The MVE mean for individuals with the GG genotype at rs943580 is lower than for individuals with 1 or 2 A alleles and a formal comparison of the 2 groups (GG versus [AG+AA]) was highly significant (\( P=0.0005 \)). Because of the high correlation between rs943580 and rs699 and rs5051, similar results are seen for rs699 and rs5051, with individuals homozygous for the major allele having a lower mean MVE. Adjusted means (by genotype) of heart rate, systolic blood pressure, diastolic blood pressure, and stroke volume are also listed because these phenotypes may be intermediate factors on the pathway between AGT and MVE.

Table 5 lists some results from our haplotype analysis. No haplotype associations achieved a level of significance of \( P<0.005 \) in either race, and only 4 associations achieved a level of significance of \( P<0.05 \) (haplotype 4 and MVE, haplotype 6 and MVE, haplotype 3 and MWS, and haplotype 2 and RWT, all in the black population). Nonetheless, to better understand the individual SNP associations with MVE, we examined the association of all major haplotypes with MVE. Table 5 shows the results of the regression of each major haplotype individually on MVE. In these individual
TABLE 3. \( r^2 \) Calculations for Black and White Subpopulations

<table>
<thead>
<tr>
<th>rs5046</th>
<th>rs5049</th>
<th>rs5050</th>
<th>rs5051</th>
<th>rs2493134</th>
<th>rs4762</th>
<th>rs699</th>
<th>rs2493132</th>
<th>rs7079</th>
<th>rs943580</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.38</td>
<td>0.005</td>
<td>0.08</td>
<td>0.10</td>
<td>0.003</td>
<td>0.10</td>
<td>0.06</td>
<td>0.10</td>
<td>0.05</td>
<td>0.10</td>
</tr>
</tbody>
</table>

rs5046: 1 0.005 0.10 0.06 0.10 0.05 0.10
rs5049: 1 0.01 0.21 0.27 0.006 0.25 0.06 0.13 0.24
rs5050: 1 0.01 0.01 0.01 0.01 0.005 0.0009 0.003
rs5051: 1 0.77 0.005 0.85 0.13 0.33 0.71
rs2493134: 1 0.005 0.92 0.14 0.45 0.77
rs4762: 1 0.005 0.92 0.14 0.45 0.77
rs699: 1 0.14 0.49 0.85
rs2493132: 1 0.08 0.14
rs7079: 1 0.55
rs943580: 1

TABLE 4. Adjusted Means \( \pm \) SE of Echo Variables by SNP Genotype in the Black Population

<table>
<thead>
<tr>
<th>rs943580 Genotype</th>
<th>rs699 Genotype</th>
<th>rs5051 Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA (n=9)</td>
<td>AG (n=116)</td>
</tr>
<tr>
<td></td>
<td>TT (n=10)</td>
<td>CT (n=120)</td>
</tr>
<tr>
<td></td>
<td>GG (n=8)</td>
<td>AG (n=107)</td>
</tr>
</tbody>
</table>

MVE* 89.1 \( \pm \) 5.8 86.1 \( \pm \) 1.7 79.5 \( \pm \) 1.0
Heart rate† 68.8 \( \pm \) 3.8 72.4 \( \pm \) 1.1 69.0 \( \pm \) 0.7
SBP† 117.8 \( \pm \) 6.7 131.1 \( \pm \) 1.8 126.8 \( \pm \) 1.1
DBP† 70.7 \( \pm \) 3.3 75.5 \( \pm \) 1.0 73.7 \( \pm \) 0.7
Stroke volume† 76.8 \( \pm \) 5.4 76.3 \( \pm \) 1.4 76.0 \( \pm \) 0.9

*Means are adjusted for age, sex, heart rate, and hypertension status; † means are adjusted for age, sex, and hypertension status.
suggest the difference in diastolic stiffness and active relaxation between genotypes was not explained solely through AGT-mediated changes in these intermediate traits. However, we recognize that these intermediate factors were measured only once and may not reflect the complete hemodynamic history of the study participants. An alternative explanation of the AGT genotype relation to MVE is that AGT has a direct action on the relaxation of the myocardium. In contrast, the absence of associations in this study with LV mass, RWT, and LVIDD suggests that AGT plays less of a significant role in simple growth and proliferation of the heart muscle. This is in contrast to 2 previous studies that found a significant association between the rs699 SNP and LV mass in Japanese hospital patients and Finnish endurance athletes. A possible explanation for this lack of replication is that our analysis was not sufficiently powered. However, our post hoc analysis suggests that we had 80% power in the white population to detect an association that accounted for only 5% of the trait variance, indicating our ability to detect associations of fairly modest effect size. Another possibility for the lack of replication is heterogeneity in the causes of various LV traits between different study populations. Within our own study population, we found very different association results in the white and black samples. Such results are not unexpected given the density of linkage disequilibrium patterns (evidence of a possible selective sweep in non-African populations) and different hypothesized selective pressures on sodium retention between tropical and temperate populations.

Although one would hope that haplotype analysis would refine the results of a multiple SNP association study, in this study, we actually obtained smaller *P* values from the single SNP analysis. The adjusted means for individuals homozygous for several of the major haplotypes may have reduced our ability to predict accurate adjusted means of MVE for several of these haplotypes.

Our study had several strengths. This was one of the first studies to examine the association of echocardiographic phenotypes other than LV mass with AGT SNPs. In addition, our study conducted analyses in black and white populations, which is important given the different linkage disequilibrium structures that are present in the different populations. There were also limitations to our study. Setting a threshold of *P* < 0.005 for a significant result, although necessary to control for multiple testing, also limited our power to detect small associations of AGT SNPs with LVH phenotypes. In addition, the tight linkage disequilibrium structure of the AGT gene made it difficult to identify which SNP was the causal SNP in the association. Previous studies have suggested that associations seen with rs699 are actually attributable to the promoter SNP rs5051. The most significant SNP in our analyses, rs943580, is in high linkage disequilibrium with the rs5051 and rs699 SNPs, but neither the association of rs5051 or rs699 and MVE reached our preset level of significance. Had the SNPs been in less tight linkage disequilibrium, haplotype analyses might have helped to determine the causal SNP. However, because of the extremely tight linkage disequilibrium within the gene, haplotypes with atypical combinations of AGT SNPs are too rare to be analyzed. If the MVE/AGT association holds up in further population studies, laboratory experiments may be necessary to determine the causal SNP in the association.

**Perspectives**

In this study, 1 SNP in the AGT gene was found to be associated with MVE in blacks. Previous findings that SNPs in the AGT gene are associated with LV mass were not replicated. Because of the high linkage disequilibrium in certain regions of the AGT gene, it is difficult to determine whether rs943580 is the causal SNP or if the causal SNP is rs5051, a promoter SNP that is known to be associated with hypertension. Further studies need to be performed to validate this result and to determine the true causal SNP in the analysis.

**Acknowledgments**

HyperGEN is funded by National Heart, Lung, and Blood Institute (NHLBI) R01 HL55673 and cooperative agreements (U10) with NHLBI: HL54471 (UT FC), HL54472 (MN Laboratory), HL54473 (DCC), HL54495 (AL FC), HL54496 (MN FC), HL54509 (NC), and HL54515 (UT DNA Laboratory). L.J.R.-T. is supported in part by NHLBI training grant T32 HL07972. The authors are grateful for...
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Hypertension. 2005;46:1294-1299; originally published online November 14, 2005; doi: 10.1161/01.HYP.0000192653.17209.84
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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