Combinations of Variations in Multiple Genes Are Associated With Hypertension


Abstract—The genetic analysis of hypertension has revealed complex and inconsistent results, making it difficult to draw clear conclusions regarding the impact of specific genes on blood pressure regulation in diverse human populations. Some of the confusion from previous studies is probably due to undetected gene-gene interactions. Instead of focusing on the effects of single genes on hypertension, we examined the effects of interactions of alleles at 4 candidate loci. Three of the loci are in the renin-angiotensin-system, angiotensinogen, ACE, and angiotensin II type 1 receptor, and they have been associated with hypertension in at least 1 previous study. The fourth locus studied is a previously undescribed locus, named FJ. In total, 7 polymorphic sites at these loci were analyzed for their association with hypertension in 51 normotensive and 126 hypertensive age-matched individuals. There were no significant differences between the 2 phenotypic classes with respect to either allele or genotype frequencies. However, when we tested for nonallelic associations (linkage disequilibrium), we found that of the 120 multilocus comparisons, 16 deviated significantly from random in the hypertensive class, but there were no significant deviations in the normotensive group. These findings suggest that genetic interactions between multiple loci rather than variants of a single gene underlie the genetic basis of hypertension in our study subjects. We hypothesize that such interactions may account for the inconsistent findings in previous studies because, unlike our study, prior studies almost always examined single-locus effects and did not consider the effects of variation at other potentially interacting loci. (Hypertension. 2000;36:2-6.)

Key Words: hypertension, essential genes renin-angiotensin system receptors, angiotensin II angiotensinogen blacks ethnic groups

The genetic basis of essential hypertension is complex. Although variations in a variety of genes have shown an association with hypertension in some studies, these associations are often not reproducible in studies of other populations. For example, an ACE insertion/deletion (I/D) polymorphism showed an association with hypertension in African Americans but not in Europeans.1–4 This inconsistencies is explained, at least in part, by the subsequent finding that the I/D polymorphism is in linkage disequilibrium (LD) with 17 other single nucleotide polymorphisms (SNPs) in the ACE gene.5 Some of these SNPs may be of functional significance and their frequencies may vary significantly between different ethnic groups. In another example, an angiotensinogen (AGT) coding polymorphism (M235T) showed an association with hypertension in several studies of Europeans and Japanese but not in studies of African Americans.6–8 Finally, although some angiotensin II type 1 receptor (AT,R) variants showed an association with hypertension in a Finnish study,9 differences in population frequencies of these alleles have made assessment of their association with hypertension in African Americans difficult.10,11

Using a different approach, we examined the contributions of simultaneous variations in several genes. Our results suggest that combinations of alleles at a variety of candidate loci may be more important than the variants that are found at any single locus in causing hypertension. We suggest that inconsistent results from previous association studies on various candidate genes with hypertension may have resulted from differences in the polymorphism frequencies at several loci simultaneously in the different study populations. These differences in the underlying genetic architecture of the study populations cause the relative contributions of any given

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variant to vary from 1 population to another. Thus, we hypothesize that the combined interactions between alleles at different loci may associate with hypertension despite the lack of significant association with variants at any single locus. On the basis of our findings, we propose that multiple variants at multiple loci may be in highly significant disequilibrium with each other in either the control or disease classes, indicating interactions among these or linked genes. Thus, we infer that if LD is found in the disease class, but not in the control class, the interactions are likely to be associated with the disease phenotype. Our model is a simple extension of current ideas on the use of LD between a marker and a disease gene as a tool to identify genes that predispose individuals to common diseases, and it is supported by the results of cited studies, as well as our data.12

In our analysis of genotypes at multiple loci in hypertensive and control Ghanaians, we show that combinations of nonallelic interactions between variant alleles at multiple candidate loci exhibit associations with hypertension even though significant single-locus effects are not detected. In the present study, we studied a population from Accra, Ghana. Genomic DNA samples were obtained from nonrelated individuals who had their phenotype determined regarding blood pressure status (see later) and genotyped for previously identified variants, and the genotypes at each locus were examined separately and in combination for association with hypertension. The variants were in genes comprising the renin-angiotensin-aldosterone pathway, including ACE, AGT, and AT1R, which have been associated with hypertension in some populations, and 3 SNPs at a fourth locus named FJ.13

Methods

Human Subjects
A total of 177 subjects (51 normotensives and 126 hypertensives) were recruited during November and December 1996 and July and August 1997 in Accra, Ghana. Recruitment was done at the clinics of the Department of Medicine at the Korle Bu Teaching Hospital of University of Ghana Medical School (18 normotensives and 24 hypertensives), the Mamprobi Hypertension Clinic (17 normotensives and 89 hypertensives), and the Kaneshie Market (16 normotensives and 13 hypertensives). Subject recruitment protocols were independently analyzed for hypertensive and normotensive classes did not differ significantly in either mean (P=0.15) or variance (P=0.93). Note that the age of diagnoses were determined from the medical records of most hypertensives, of whom the vast majority (89 of 126) were recruited from the Mamprobi clinic, where these records have been maintained for ~2 decades. Also, because results in male and female subjects did not differ from each other in preliminary analyses, they were combined in the presented analyses.

DNA Isolation and Analysis
Blood samples (6 to 12 mL) were collected from each consenting subject into purple top (EDTA) tubes. DNA was isolated from blood samples with use of the Puregene kit from Gentra Systems and according to the manufacturer’s protocol. Aliquots of genomic DNA were genotyped for each of the above-mentioned polymorphisms with standard PCR or PCR/RFLP methods for these polymorphisms.9,13–16

Results and Discussion

Single-locus effects were examined by comparing allele and genotype frequencies of each of the 7 variants at the 4 candidate loci. No significant effects were found for any allele or genotype frequencies at any 1 locus (Table 2). Our findings suggested that none of these sites alone showed significant association with hypertension. Although this might be a stopping point of a standard analysis, we further examined the data to detect possible multiple-locus interactions. To test for genetic interactions, the hypertensive and normotensive populations were independently analyzed for single- and multiple-locus deviations from H-W or linkage equilibrium.19,20 H-W equilibrium analysis tests whether alleles at the same locus are randomly combined with each other to form genotypes within each of the populations. Deviations from equilibrium, especially across loci, may associate with hypertension despite the lack of significant association with variants at any single locus (Table 2). Our findings suggested that none of these sites alone showed significant association with hypertension. Although this might be a stopping point of a standard analysis, we further examined the data to detect possible multiple-locus interactions. To test for genetic interactions, the hypertensive and normotensive populations were independently analyzed for single- and multiple-locus deviations from H-W or linkage equilibrium.19,20 H-W equilibrium analysis tests whether alleles at the same locus are randomly combined with each other to form genotypes within each of the populations. Deviations from equilibrium, especially across loci, may serve as indicators that the combination of alleles at different loci either increases or decreases disease susceptibility. Given that the 4 loci under study are each on different autosomes (the ACE, AGT, AT1R, and FJ loci are on chromosome 17, 1,
3, and 4, respectively), any deviations would support a role for multilocus or nonallelic interactions.

One hundred twenty multilocus comparisons were made for each phenotypic class. Interestingly, 16 multilocus combinations deviated significantly from equilibrium in the hypertensive class (Table 3), and an additional 10 comparisons had a probability value of 0.05 to 0.10 (Table 3). In addition, 2 loci were individually out of H-W equilibrium in the hypertensive class (ACE $P = 0.013$ with an inbreeding coefficient $f = 0.22$, and AT$_1$R $P = 0.044$ with $f = 0.39$). In contrast, no comparisons in the normotensive class deviated significantly from H-W expectations at any locus or from equilibrium among loci; the lowest multilocus comparison $P$ value for this class was 0.24. To not bias multilocus comparisons by the single-locus H-W disequilibria, H-W analyses with ACE and AT$_1$R were conditioned on genotype frequency.

The number of significant comparisons in the hypertensive class is even clearer when the analysis is subdivided into classes on the basis of the number of sites compared. Table 4 shows that most of the significant results were in the 2- and 3-site comparisons. The number of significant results far exceeds what would be expected by chance alone in these 2 classes, with 6 and 8 significant comparisons for the 2- and 3-site cases, respectively, when only 1 or 2 significant results are expected.

To exclude the possibility that differences in sample sizes may contribute to the finding of differences between

### Table 2. Single-Locus Allele Frequencies and Genotype Distributions

<table>
<thead>
<tr>
<th>Locus</th>
<th>Hypertensive</th>
<th>Normotensive</th>
<th>Frequency</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>T147 M</td>
<td>106</td>
<td>47</td>
<td>2 0</td>
<td>0.99</td>
</tr>
<tr>
<td>M235T</td>
<td>92</td>
<td>43</td>
<td>18 0</td>
<td>0.92</td>
</tr>
<tr>
<td>ACE$^{II}$</td>
<td>14</td>
<td>11</td>
<td>74 38</td>
<td>0.40</td>
</tr>
<tr>
<td>AT$_1$ Receptor</td>
<td>113</td>
<td>45</td>
<td>3 1</td>
<td>0.98</td>
</tr>
<tr>
<td>FJ Site 1</td>
<td>31</td>
<td>12</td>
<td>64 29</td>
<td>0.51</td>
</tr>
<tr>
<td>FJ Site 2</td>
<td>12</td>
<td>8</td>
<td>62 20</td>
<td>0.38</td>
</tr>
<tr>
<td>FJ Site 4</td>
<td>99</td>
<td>42</td>
<td>21 0</td>
<td>0.88</td>
</tr>
</tbody>
</table>

*Number of times the 0.05 level was obtained in 10 random samples from 51 individuals from the hypertensive class.
†Number of times the 0.10 level was obtained in 10 random samples from 51 individuals from the hypertensive class.
‡Proportion of times analyses from the resampled group obtained 0.05 or 0.10 level of significance.

### Table 4. Relative Contribution of Multilocus Comparisons

<table>
<thead>
<tr>
<th>No. of Loci</th>
<th>No. of Comparisons</th>
<th>No. of Significance Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Sites</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>3 Sites</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>4 Sites</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>5 Sites</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>6 Sites</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>7 Sites</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>16</td>
</tr>
</tbody>
</table>

3-site cases, respectively, when only 1 or 2 significant results are expected.

To exclude the possibility that differences in sample sizes may contribute to the finding of differences between
the hypertensive and normotensive classes, additional analyses were made. Data were used again from 51 hypertensive subjects sampled without replacement from the total hypertensive class, and the LD analysis was repeated on the subset of the data. The sampling was repeated 10 times, and the number of times the analysis resulted in a 0.05 or 0.10 level of significance is shown in Table 3. All of the 16 original significant results were repeated at least once, and all except 1 of the 10 original results found at the 0.10 level were also repeated at least once, with the more significant results seeming to be significant more often in the resampling analysis (Table 3). Although many of the results were repeated less than half of the time, these results still support the conclusion that the hypertensive and normotensive classes differ with respect to multilocus interactions, because disequilibrium was commonly found in the hypertensive class in the reanalyses. For all of these subsamplings, an average of 6.6 significant comparisons at the 0.05 level and 6.9 at the 0.10 level were found in these 10 samples of the hypertensive class. These results reinforce the conclusion that these 2 groups differ from each other, although the exact nature of the difference requires further study.

The amount of disequilibrium observed in the hypertensive class is high compared with values generally found in human populations for unlinked loci. In fact, a previous study of 2 locus interactions with hypertension in a Chinese population found no such interactions among 6 loci on 4 chromosomes, although the authors studied only 1 of our 4 loci (AGT). These observations, in conjunction with the lack of disequilibrium in the normotensive class, suggest that interactions between candidate sites contribute to the cause of the disease in this Ghanaian cohort. The deviation from equilibrium in so many instances is particularly striking because the 4 candidate genes are unlinked and the a priori predictions of disequilibria are not likely to extend beyond a few kilobases, much less across linkage groups. Alternatively, the differences in disequilibrium that we observe, as well as similar results in other association studies, could be caused by stratification between the cases and control subjects. We believe this is unlikely in present study because we did not find any single-locus effects or any disequilibrium in the normotensive class. Stratification of nonrelated control subjects drawn from the same population sufficient to selectively mask all significant H-W disequilibria in that group alone seems unlikely given the criteria we used for subject selection. Nevertheless, we plan to test the possibility of selective stratification of our hypertensive or nonhypertensive study groups with the use of microsatellite markers unlinked to hypertension candidate genes.

The present study strongly suggests the potential importance of gene-gene interactions in the cause of hypertension. Furthermore, our data demonstrate the use of candidate gene disequilibrium analysis as a method of analyzing multilocus phenomena. Our results also suggest that many of the negative results for single-locus studies of hypertension may be due to the fact that interactions among multiple genes that are components of complex homeostatic systems predispose individuals to high blood pressure rather than the isolated effects of single genes in a single pathway. Testing of the generality of this finding will require that studies be undertaken in other populations. These kinds of multilocus interactions would also be consistent with the complex inheritance of hypertension.

Acknowledgments

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