**A Genome-Wide Linkage Analysis**

**Investigating the Determinants of Blood Pressure in Whites and African Americans**

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Evidence for genomic regions influencing systolic and diastolic blood pressure (BP) were assessed in a whole genome linkage analysis in 211 African American and 160 white families as part of the GenNet network of the National Heart, Lung and Blood Institute-sponsored Family Blood Pressure Program. Multipoint regression and variance components linkage methods were used to analyze 372 polymorphic markers. Statistically compelling evidence for linkage ($P$ values .0057 and .00023, respectively) was found on chromosome 1. Our results support the idea that BP regulation is most likely governed by multiple genetic loci, each with a relatively weak effect on BP in the population at large. Am J Hypertens 2003;16:151–153 © 2003 American Journal of Hypertension, Ltd.

**Key Words:** Hypertension, blood pressure, genetics, heredity, linkage analysis.

Several rare genes have now been identified that predispose rare individuals to high blood pressure (BP) and severe hypertension. Liddle’s syndrome, glucocorticoid-remedial aldosteronism, and the syndrome of apparent mineralocorticoid excess all have single gene determinants and are inherited in a simple Mendelian fashion.1–5 Polymorphic variation in the genes responsible for these Mendelian forms of hypertension, so far, do not appear to play a significant role in more common forms of the disease. As a result, researchers have undertaken genome-wide searches for novel genes that could influence common forms of hypertension, as well as BP regulation.6

The heritability of BP (ie, the proportion of variation in BP that is due to genetic factors) has been estimated in several different populations studies as being between 15% and 60%, suggesting that genome-wide searches using polymorphic markers have some potential for success. However, the number, genomic location, and ultimate effect of actual BP-related genetic factors remain largely unknown. Recent genome-wide searches for hypertension and BP influencing genes have found evidence for genomic regions on many chromosomes, but are not consistent across studies.7–9 This lack of consistency may be due to the variation in the trait definitions used in these studies (eg, hypertension status, BP level, extreme BP levels, medication use), genetic heterogeneity between the different populations studied, or the use of different data analysis methods.

Therefore, to identify genomic regions that influence BP variation and hypertension susceptibility, we performed a genome-wide linkage analysis in two large samples, one composed of African Americans and one composed of whites, using systolic and diastolic BP as the phenotype of interest. Two analytic approaches based on variance components and regression models were used in an effort to capitalize on the strengths of each method.

**Methods**

**Study Populations**

Families were recruited through mostly unmedicated probands with elevated BP levels who were younger than the age typically associated with hypertension onset. A discussion of the sampling design for the present study is available elsewhere.10 The white sample was ascertained in Ann Arbor, Michigan.

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from Tecumseh, MI. Records on families involved in previous studies in Tecumseh were used to identify probands between 18 and 50 years old. Within this group, eligibility was defined as having a recorded systolic BP in the upper 25% of the BP distribution using gender-specific criteria and no use of antihypertensive medication. The African American participants were recruited from Maywood, IL, near metropolitan Chicago. A prior study was used to identify probands 25 to 45 years of age with a recorded systolic BP in the upper 20% of the distribution. Siblings and parents of the probands in both samples were recruited without regard to BP or hypertension status. All subjects gave written, informed consent before participation in the study.

The BP measurements were made using a standardized protocol with staff from each data collection site participating in joint training and certification. Two manual measurements were taken on each subject with a standard mercury manometer and the average systolic and average diastolic values of these measurements were used as the phenotype of interest in this study. The subjects answered a detailed questionnaire about medical history, risk factors, and medication use. Pedigree information was obtained from all participants and verified by examination of birth certificates in Maywood.

Genotyping

Genotyping was performed by the National Heart, Lung and Blood Institute Mammalian Genotyping Service (Dr. James Weber, Director) at the Marshfield Clinic, Marshfield, WI (http://research.marshfieldclinic.org/genetics). A standard set of 372 DNA markers (set 8) on the 22 autosomes were genotyped on 1234 individuals. The number of families in the African American sample with phenotype and genotype data totaled 211 including 303 sibpairs and 211 half-sibpairs. The final white sample was composed of 160 families with 386 sibpairs and 8 half-sibpairs.

Statistical Analysis

The variance components module within the GENEHUNTER2 software program\(^\text{11}\) was used to estimate, by maximum likelihood, mean BP values by gender, the proportion of BP variation attributable to each tested locus, the proportion of BP variation attributed to residual polygenic (ie, nonmajor locus specific) effects, and the proportion of variation attributable to random environmental effects. Age was also included as a covariate in the analysis. Parameter estimates were calculated at 1-cM intervals along each chromosome. All pairs of phenotyped sibs were included in the analysis, and these were weighted to account for the statistical dependence among all pairs of siblings within a single family. The Sibpal2 module of the SAGE computer package\(^\text{12}\) assumes a regression-based method in which the mean-corrected cross-products of the sibpair trait values are regressed on the proportion of alleles that these pairs share identical-by-descent (IBD). A generalized least squares regression is performed to allow for correlations between the pairs of sibpair cross-products. Two covariates, age and gender, were included in the analysis, and these covariates were calculated as the mean corrected cross-products of the sibpairs’ age and gender values. The total genetic variance (additive plus dominance) of the phenotypes attributable to a genetic locus was estimated at 1-cM intervals. The estimated parameters in the regression were tested for significance using a \(t\) statistic. This method is less sensitive to departures from normality in the trait distribution than the variance components method. However, in small samples, the Sibpal2 test statistic may not follow a \(t\) distribution and can result in \(P\) values that are liberal. To overcome this, a simulation method was used to compute the empirical \(P\) value for all chromosomal regions that were significant by the asymptotic \(t\) test.

Results

Table 1 shows the most significant results. The highest likelihood score (LOD) obtained in the GENEHUNTER variance components analysis, and the only one to reach the suggestive level for evidence of linkage\(^\text{13}\) was found in the white families with diastolic BP as the phenotype. This LOD score of 2.96 (equivalent to a \(P\) value of .00023) was detected on chromosome 1 at 170 cM (from pter) just distal to marker GGAA5F09. The only other LOD score

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**Table 1.** GENEHUNTER2 maximum LOD scores and SAGE Sibpal2 minimum \(P\) values

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>African American SBP</th>
<th>African American DBP</th>
<th>White SBP</th>
<th>White DBP</th>
<th>Sibpal 2 African American SBP</th>
<th>Sibpal 2 African American DBP</th>
<th>Sibpal 2 White SBP</th>
<th>Sibpal 2 White DBP</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>.45</td>
<td>.82</td>
<td>.16</td>
<td>2.96</td>
<td>NS</td>
<td>.019</td>
<td>NS</td>
<td>.0057</td>
</tr>
<tr>
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<td>.27</td>
<td>.44</td>
<td>1.11</td>
<td>1.33</td>
<td>.019</td>
<td>.031</td>
<td>.008</td>
<td>.0004</td>
</tr>
<tr>
<td>11</td>
<td>.22</td>
<td>.67</td>
<td>1.66</td>
<td>.15</td>
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<td>NS</td>
<td>.014</td>
<td>NS</td>
</tr>
<tr>
<td>12</td>
<td>.18</td>
<td>.27</td>
<td>.00</td>
<td>.15</td>
<td>NS</td>
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<td>NS</td>
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<tr>
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<td>.19</td>
<td>.45</td>
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<td>.22</td>
<td>.012</td>
<td>.014</td>
<td>NS</td>
<td>.00015</td>
</tr>
</tbody>
</table>

SBP = systolic blood pressure; DBP = diastolic blood pressure; NS = not significant. Explanation of bold lettering in text.
more than 1.5 was for systolic BP in the white families on chromosome 11, which was 1.66 at 76 cM.

The Sibpal2 results are shown on the right side of Table 1. Empirical \( P \) values obtained from simulation (computed only for those regions having asymptotic \( P \) values of <.001) are shown in bold. The region with the greatest evidence for linkage using the regression approach was on chromosome 3 in white families for diastolic BP with a \( P \) value of .0004 at 119 cM at marker GATA68D03. Linkage evidence was also detected on chromosome 1 at 168 cM for diastolic BP in whites with an empirical \( P = .0057 \) at the same location determined to be significant via the GENEHUNTER2 analysis.

**Discussion**

On the basis of our genome-wide linkage analysis, we find some evidence for a BP regulation locus on chromosome 1 in whites. The area on chromosome 1 with the greatest evidence for linkage using both analytic methods is lacking in known genes that appear to have an influence on BP. The renin and angiotensinogen genes both reside on chromosome 1q, and these loci have been associated with hypertension susceptibility. However, both lie distal to the region giving us the most evidence for linkage. The natriuretic peptide precursors A and B appear to influence BP and are located on chromosome 1p36.2 but again, are not close enough to the candidate regions.

It is widely acknowledged that BP variation is most likely the result of a combination of many genetic and environmental factors and, therefore, it is not surprising that we have found only moderate support for linkage in our large sample of families. Although the variance components method implemented in GENEHUNTER2 is in large part similar to the Sibpal2 regression method, each method has particular strengths and weaknesses that could produce different results in the same dataset. For example, the large signals on chromosomes 3, 12, and 18 using Sibpal2 were not reproduced in the GENEHUNTER2 analysis. One possible reason for this could be because the regression method in Sibpal2 estimates the total genetic effect (additive plus dominance variance) explained by the locus, whereas in the GENEHUNTER2 method only the additive genetic effect was estimated.

The lack of consistency in the linkage results on chromosome 1 between the white and African American samples could be due to heterogeneity of the BP level determinants, such that a trait locus in this region only has an effect on BP in certain individuals who have a particular genetic background. However, even within the samples there is likely to be different genes contributing to BP variation. We are pursuing analyses that cluster together families that have a larger degree of sharing over their entire genome in the hopes that this will produce groups of families that are more similar overall and that may have a common genetic etiology.

If a small number of individual loci are acting in an additive fashion on BP variation, the effects may be large enough to be detected using linkage analyses of the type we pursued. However, if there are many loci involved, each with small effects that may act additively or epistatically, the methods we applied may not have enough power to detect any individual locus effects. Under a model in which it is assumed that loci with small effects are operative, it will be difficult to determine what signals from the linkage analysis are large enough to consider possible trait influencing loci.\textsuperscript{14,15} Although the linkage on chromosome 1 in the white sample may well be a false-positive signal, because this area gave the largest evidence for linkage using two different analysis methods, we consider it worth investigating further.

**References**


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