Identification of Hypertension-Related QTLs in African American Sib Pairs


Abstract—To link hypertension-related phenotypes with chromosomal loci, genome scans were performed in 150 African American sib pairs concordant for essential hypertension. Phenotypes included blood pressure, anthropomorphic measurements, and estimates of body fluid compartments as determined by impedance plethysmography. These phenotypes were also measured in 335 normotensive African Americans. Phenotypes with LOD scores >3.3 were further evaluated for significance by use of permutation procedures. Significant linkage was detected for body mass index (BMI) on chromosomes 1 and 8 and for the ratio of extracellular water to total body water (ECF/TBW) on chromosomes 3, 5, 6, and 7. Both BMI and ECF/TBW were greater in hypertensive sibs than in normotensive subjects (P<0.001). In a subset of hypertensive sibs and normotensive subjects, average 24-hour blood pressures were correlated with ECF/TBW (P<0.01). A region linked to BMI in the hypertensive sibs corresponds to a region of conserved synteny containing blood pressure–related QTLs in an F2 cross of Brown Norway×Dahl salt-sensitive rats. Focusing on hypertension-related phenotypes is a promising approach for identifying the genetic determinants of hypertension. (Hypertension. 2002;40:634-639.)

Key Words: blacks ■ body mass index ■ comparative mapping ■ genome scan ■ plethysmography

Adoption, twin, and family studies document a significant heritable component to blood pressure levels and to hypertension. Family studies controlling for a common environment indicate that blood pressure heritability is in the range of 15% to 35%.1–3 In twin studies, heritability estimates of blood pressure are ≈60% for men and 30% to 40% for women.3–5 Nevertheless, genetic studies of hypertension in human populations remain challenging because of the likely multiplicity of contributing genes, the modest nature of gene effects, and the genetic heterogeneity among populations.

One fruitful approach to identifying genes of hypertension included studies in a limited number of families with early onset of hypertension and other characteristic phenotypes.6 However, the relevance of these genetic variants to hypertension in the general population remains to be determined. Association study approaches have included the study of candidate genes and genes identified from whole-genome linkage scans. Polymorphisms within a relatively large number of candidate genes have been tested for association with hypertension, and the results have been inconsistent.7

The development of thousands of microsatellite genetic markers has made whole-genome scans feasible. There are several recent reports of genome-wide scans in diverse populations attempting to link either blood pressure itself or a clinical diagnosis of hypertension to specific chromosomal loci.8–19 These studies have included sib pairs concordant for hypertension, as well as siblings who are extremely divergent for blood pressure. Although evidence for linkage of blood pressure with specific chromosomal regions has been observed in individual studies, results have not been consistent across studies. The lack of consistency among studies may reflect the fact that blood pressure is a difficult phenotype to assess because of its minute-to-minute variation and because of the potential confounding effects of antihypertensive medications. In addition, blood pressure is the culmination of interactions among autonomic, cardiovascular, renal, and endocrine control systems. Each of these systems may be influenced by different genes and different environments.

Our long-term goal is to focus on the physiological pathways that determine arterial pressure. In an effort to link hypertension-related phenotypes with specific chromosomal loci, total-genome scans were performed in African American sib pairs concordant for hypertension. Studies were performed in African Americans because of the high prevalence of hypertension and hypertension-related cardiovascular disease in this ethnic group.20 The purpose of this report is to describe the results of the linkage analyses of outpatient phenotypes obtained in the affected sib pairs.

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Methods

Consenting African American sib pairs, age 18 to 55 years, with essential hypertension were potential candidates for study. Subjects were defined as African American on the basis of self-identification, birth in the continental United States, both parents reported as being African American, and English as the native language. All subjects were recruited from a variety of community resources and health providers within the Milwaukee area. Subjects were considered to have hypertension if standardized measurements of blood pressure were >140/90 mm Hg on 2 occasions or if they were taking antihypertensive medications. Exclusion criteria included diabetes mellitus, serum creatinine concentration >2.2 mg/dL, substance abuse (including alcohol), and mispaternity.

Outpatient phenotypic measurements included standardized measurements of blood pressure, anthropomorphic measurements (height, weight, waist and hip circumferences, skinfold thicknesses at multiple sites), and estimates of body fluid compartments determined by bioelectrical impedance plethysmography. At the time these measurements were obtained, 66% of the hypertensive subjects were taking antihypertensive medications. Percent body fat was determined from measurements of skinfold thickness. Blood pressure measurements were obtained with subjects seated quietly for at least 10 minutes. Three measurements were obtained in each arm, 2 minutes apart, with a mercury sphygmomanometer and a cuff calibrated for arm girth. The recorded blood pressure value is an average of the 3 measurements in the arm with the higher blood pressures. All personnel who measured blood pressures were trained and certified by use of the Shared Care method.

Bioelectrical impedance measurements were obtained using an instrument developed in the Department of Physiology at the Medical College of Wisconsin. The whole-body impedance bridge consists of a constant current generator (280 microamps), a differential input amplifier, a precision rectifier circuit, signal conditioning circuits, and a digital display of impedance. The system is battery-operated, from two 12-volt rechargeable sealed lead-acid batteries connected to the LM340T8 and LM320T8 voltage regulators to provide 8 and ~8 volt power for the circuitry. The system measures impedance within ±5% of the measured values, and is functional between 50 and 1000 ohms, which exceeds the range for whole-body impedance in human subjects. Measurements were obtained with subjects in the seated position, and electrodes were applied to the right wrist and the left ankle. For each subject, impedance was measured at frequencies of 1, 10, and 100 kHz. The phenotype evaluated in the linkage analysis is the ratio of resistance measured in response to a current applied at a low frequency (10 kHz) divided by the resistance to current applied a high frequency (100 kHz). This provides an estimate of the relationship of extracellular fluid volume to total body water. Measurements were obtained in triplicate and averaged.

After the blood pressure and impedance measurements, peripheral venous blood was obtained in the nonfasting state for measurement of serum cholesterol, creatinine, and glucose concentrations and for extraction of DNA. A detailed family history was also obtained.

Overall, outpatient phenotypic measurements and genome scans were performed in 150 affected sib pairs. For phenotypic comparisons between hypertensive and normotensive individuals, identical measurements were obtained in 335 normotensive African Americans. Subsequently, 24-hour blood pressure monitoring was performed in 65 of the hypertensive sib pairs and 69 of the normotensive subjects on an inpatient Clinical Research Center after antihypertensive medications had been withdrawn for at least 1 week. Measurements were obtained with an Accutrac II monitor (Suntech Medical Instruments Inc) every 20 minutes during the day (5:00 AM to 11:00 PM) and every 45 minutes during the night (11:00 PM to 5:00 AM). To assure a wide blood pressure separation between hypertensive and normotensive subjects, normotensive individuals selected for the inpatient study were drawn from the lower third of the population-based blood pressure distribution, based on National Health and Nutrition Examination Survey (NHANES) III data. All subjects signed informed consent forms, and each subject received a $25 stipend for outpatient testing and a $200 stipend for completing the inpatient study. The protocols were approved by the Medical College of Wisconsin Human Research Review Committee.

Whole-genome scans were performed under the supervision of Dr Eric Lander at the Whitehead Institute for Biomedical Research at Massachusetts Institute of Technology. Genome scans were completed in the hypertensive sib pairs, using 363 microsatellite markers, and linkage was evaluated by use of the Genehunter computer program. The distances between markers averaged 9.2 CM across the genome (Genethon). The average heterozygosity across all markers on all chromosomes was 0.78, with a range from 0.75 (chromosome 11) to 0.81 (chromosome 15). For each marker, allele frequency was estimated by counting the alleles held by original founders in the pedigree. This method is implemented in MAPMAKER/PEDMANAGER 0.9 and tends to not overestimate the sharing between relatives. Before the Genehunter analysis, each phenotypic trait was tested for normality by inspection of quintile plots and confirmed with the Kolmogorov-Smirnov test before or after log or square-root transformation. The variance component analysis module in Genehunter was used to evaluate linkage. Linkage analyses were performed both with and without adjustment for covariates (age, gender, body weight), and the results did not differ appreciably (data not shown). Consequently, only results with adjustment for covariates are presented.

Lander and Kruglyak published criteria for the significance of a LOD score in genome scans. However, these calculations were based on assumptions not explicitly found in most studies (eg, infinitely dense map of markers), and they do not take into account the intrinsic properties of a particular data set. One robust method, which aims to provide realistic assessment of significance by incorporating the actual marker density used in a study, the marker informativeness, and the pedigree structure for the data set with regard to each phenotype, uses simulation and permutation procedures. Using these approaches, we generated “simulated” data sets by randomly permuting the given phenotypic values among the sib pairs. For each phenotype with a LOD score >3.5 (corresponds to $P<0.0002$ based on a point-wise probability value), we performed 130 genome scans with the permuted phenotype data sets to determine the threshold LOD score that would need to be exceeded for significant linkage ($P<0.01$ based on a genome-wide probability value). The criteria for assessing significance were based on the actual number of sib pairs studied. A 2-tailed $t$ test (or 1-sided $t$ test for blood pressure) was used to test statistical significance of differences in phenotypes between hypertensive and normotensive subjects. Linear regression, using the Pearson correlation coefficient, was used to evaluate the association of specific phenotypes with blood pressure. Pearson correlations were computed for the combined singleton and sib pairs by use of the within-pair correlation to appropriately weight the contribution of the sib pairs to the whole. In addition, including only 1 of the 2 hypertensive sibs from each sib pair did not change the relationships observed when both sibs were included. When indicated, ANOVA was used to test the significance of 3 group comparisons. Results are expressed as mean±SE, and $P<0.05$ was considered statistically significant.

Results

Average outpatient blood pressures in the hypertensive sib pairs were greater than blood pressure of the normotensive subjects (Table 1). Average body mass index (BMI), body surface area, percent body fat, and serum cholesterol of the hypertensive sib pairs were all significantly greater than were respective values of the normotensive subjects ($P<0.001$). Serum creatinine concentrations did not differ in the 2 groups. The impedance ratio of the hypertensive sibs was less than that of the normotensive subjects ($P<0.001$), indicating that the ratio of extracellular fluid volume to total body water (ECF/TBW) was greater in the hypertensive sib pairs. There was no association between the impedance ratio and use of antihypertensive medications. The ratio did not differ in the
following 3 groups of hypertensive subjects: (1) no antihypertensive therapy, (2) an antihypertensive regimen that included a diuretic, (3) antihypertensive agents other than a diuretic. In the hypertensive sib pairs, as well as in the combined group of hypertensive sibs and normotensive subjects, there was no correlation between BMI and the impedance ratio, indicating that these are 2 unrelated phenotypes.

Of the hypertensive sib pairs and normotensive subjects evaluated as inpatients, average 24-hour blood pressures (off antihypertensive medications) were 144±2/86±1 and 117±1/68±1 mm Hg, respectively (P<0.001). In the combined group of hypertensive sib pairs and normotensive subjects in whom the inpatient 24-hour blood pressure measurements were obtained, ECF/TBW was directly correlated with average 24-hour systolic (r=0.21; P<0.01) and diastolic (r=0.17; P<0.02) blood pressures. Among only the hypertensive subjects, ECF/TBW was not correlated with blood pressure, although among the normotensive subjects separately, ECF/TBW was significantly correlated with diastolic blood pressure, either in the combined group of hypertensive and normotensive subjects or in each group separately.

The Genehunter analysis has been completed in the 150 hypertensive sib pairs for the outpatient phenotypes (except for age) listed in Table 1. Permutation tests were performed for those phenotypes with LOD scores >3.3. Based on phenotypic-specific LOD scores for significant linkage derived from the permutation analyses, we detected significant linkage for BMI and ECF/TBW as determined by impedance plethysmography (Table 2). BMI was analyzed as a log-transformed value. Although values of the impedance ratio were not normally distributed, skewness was not significant (P=0.24), and consequently, the impedance ratio was analyzed with untransformed values. Significant linkage was not detected for any of the other phenotypes listed in Table 1. For BMI, the threshold LOD for significant linkage at the 99% confidence level was 3.27. QTLs were found on both chromosome 1 and 8 with significant LOD scores of 3.70 and 3.43, respectively (Figure 1). For the impedance ratio estimate of ECF/TBW, we identified significant linkage on chromosomes 3, 5, 6, and 7 (Figure 2). Each of these QTLs exceeded the threshold for significant linkage, as determined by permutation procedures.

### TABLE 1. Comparison of Phenotypes in Hypertensive Sibs and Normotensive Subjects

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Hypertensive Sibs</th>
<th>Normotensives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outpatient blood pressure, mm Hg</td>
<td>142±1/94±1</td>
<td>119±1/77±1†</td>
</tr>
<tr>
<td>Age, y</td>
<td>43.6±0.3</td>
<td>39.4±0.4*</td>
</tr>
<tr>
<td>Waist/hip ratio, m²</td>
<td>0.89±0.01</td>
<td>0.85±0.01</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>2.01±0.01</td>
<td>1.93±0.01‡</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>36.4±0.6</td>
<td>31.7±2.0†</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.9±0.1</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>Serum cholesterol, mg/dL</td>
<td>203±2</td>
<td>186±2†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>32.5±0.3</td>
<td>29.2±0.3‡</td>
</tr>
<tr>
<td>Impedance ratio</td>
<td>1.001±0.005</td>
<td>1.049±0.003‡</td>
</tr>
</tbody>
</table>

Values are mean±SE. Sixty-six percent of hypertensives were taking antihypertensive medications. *P<0.03; †P<0.01; ‡P<0.001.

### TABLE 2. Phenotypes That Mapped to Specific QTLs

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>LOD for Significant Linkage*</th>
<th>LOD</th>
<th>Chromosome</th>
<th>Position, cM</th>
</tr>
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<tbody>
<tr>
<td>BMI</td>
<td>3.27</td>
<td></td>
<td>3.70</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.43</td>
<td>8</td>
</tr>
<tr>
<td>Impedance ratio</td>
<td>2.42</td>
<td></td>
<td>3.94</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.49</td>
<td>5</td>
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<td></td>
<td></td>
<td>2.79</td>
<td>6</td>
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<td></td>
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<td></td>
<td>2.52</td>
<td>6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3.16</td>
<td>7</td>
</tr>
</tbody>
</table>

*P<0.01, determined by permutation procedures and based on a genome-wide P value.

Figure 1. LOD peaks for significant QTLs for BMI on chromosomes 1 and 8. The horizontal lines indicate the phenotypic-specific LOD threshold for significant linkage (P<0.01), determined by permutation procedures and based on a genome-wide probability value.
Hypertension-related QTLs in African Americans

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Discussion

Our approach to identifying the genetic determinants of hypertension has been to focus on the physiological pathways that determine arterial pressure rather than focusing exclusively on blood pressure itself. This report describes results of genetic linkage analyses of outpatient blood pressure, anthropomorphic, and bioimpedance measurements. To minimize the potential for false-positive results, we used simulation and permutation procedures for each phenotype with a LOD score >3.3 to further determine thresholds for significant linkage. The rationale for selecting this LOD threshold is based on the recommendation of Lander and Kruglyak. By using simulation and permutation procedures, we required an additional test of significance for those peaks with LOD scores >3.3.

Both because of this conservative approach for determining significance and because the relatively small number of sib pairs, significant QTLs may have been overlooked owing to limited power. In addition, the fact that 66% of the hypertensive sibs were taking antihypertensive medications may have obscured linkage of outpatient measurements of blood pressure. Nevertheless, we did observe that BMI and ECF/TBW, determined by impedance plethysmography, are significantly linked to specific chromosomal regions in these African American sib pairs. Each of these phenotypes has potentially important implications for the pathogenesis of hypertension.

In population studies, blood pressure is related to body weight, and there is an association between obesity and hypertension. Physiological mechanisms of obesity-related hypertension have recently been reviewed. We and others have suggested that obesity-associated hypertension is determined, at least in part, by genes involved in the regulation of both adiposity and blood pressure. Several candidate genes have shown linkage and/or association with obesity-related hypertension, e.g., tumor necrosis factor-α, β1-adrenergic receptor, and G protein β3-subunit. In the current study, BMI mapped to a region on chromosome 1 and a region on chromosome 8. Based on a review of the OMIM Gene Map, several genes potentially relevant to hypertension are located between the markers defining the 95% confidence interval of this QTL on chromosome 1, e.g., angiotensinogen, renin, voltage-dependent L-type calcium channels, voltage-gated potassium channel, tumor necrosis factor ligand, and 11β-hydroxysteroid dehydrogenase. Although we cannot exclude the possibility that these regions may contain obesity genes unrelated to hypertension, it has recently been reported that hypertension is linked to this same region on chromosome 1 in white families and in a small number of families with pseudohypopaldosteronism type II. Although several other chromosomal regions have been linked to obesity in different populations, these studies have not focused on the phenotype of obesity-associated hypertension. In addition, in both white and black families, genome-wide scans have not identified linkage at these locations on chromosomes 1 and 8 with BMI or other indices of obesity, in the absence of hypertension, further suggesting that these QTLs are not related to obesity per se. Alternatively, the failure of other studies to detect linkage at these QTLs on chromosomes 1 and 8 may be related to differences of study design, including study power, study population, and ascertainment strategy.

The actual parameter measured with bioelectrical impedance is the voltage that is produced between 2 electrodes located at sites different from where current is introduced. Impedance is expressed as a ratio of voltage/current and is inversely related to volume. At low frequencies, the applied current flows primarily in the extracellular space, whereas at high frequencies, the current flows through both intracellular and extracellular spaces. Thus, measurements at low frequencies reflect extracellular fluid volumes, whereas those at high frequencies reflect whole-body water, and the “impedance ratio” is an approximation of the ECF/TBW ratio.

In the current study, we observed several QTLs for the impedance ratio. The physiological relevance of this observation was confirmed by our finding that the impedance ratio was less in hypertensive than in normotensive subjects (indicating that the ECF/TBW ratio was greater in the hypertensives) and by the correlation of ECF/TBW with both systolic and diastolic blood pressures. We believe that the association of ECF/TBW with higher blood pressures likely represents an expanded extracellular fluid volume in the hypertensive subjects. Under normal circumstances, there is a direct relationship between extracellular fluid volume and blood volume, and the current observation is consistent with earlier reports estimating that >50% of African American hypertensives are salt sensitive, and that the prevalence of diuretic-sensitive (and presumably salt sensitive) hypertension approaches 75%. Salt-sensitive hypertension may be a manifestation of an expanded extracellular fluid volume.

There is limited and inconsistent information about specific genetic markers of salt-sensitivity of blood pressure. The T235 allele of the angiotensinogen gene is the most frequent allele in African Americans and may be associated with salt sensitivity of blood pressure. Polymorphisms of the ACE gene, the β1-adrenergic receptor gene, and the adducin gene have also been associated with salt sensitivity of blood pressure. Our observation that the impedance...
ratio is linked to QTLs on chromosomes 3, 5, 6, and 7 suggest that these may be promising regions to search for genes related to salt-sensitive hypertension.

With the advent of a dense rat genetic map, inbred hypertensive rat strains provide particularly robust models for the genetic dissection of various pathways that contribute to hypertension and related phenotypes. Use of inbred strains of rats removes the problem of genetic heterogeneity. Further, because the coding region of the rat genome is >90% similar to the human genome, there is a strong possibility that disease genes identified in the rat can be studied in regions of conserved gene order between humans and rats. Although the use of synteny mapping in rodent models as a comparative tool has imprecision because of the smaller number of total rat markers, recently constructed comparative genomic maps have demonstrated considerable overlap between hypertension-related QTLs in rat and human.47

In a related project,48 a total-genome scan involving F2 rats derived from a Brown Norway×Dahl salt-sensitive intercross was performed. The F2 rats were extensively phenotyped for direct or derived measures of cardiovascular, renal, and neurohormonal function. As previously reported, a number of traits were mapped to chromosomal regions with highly significant LOD scores.

Relevant to this clinical study, by comparative mapping, we evaluated potential syntenic genomic regions between rat and human for those traits that mapped to specific chromosomal regions in the human. A region on human chromosome 1 that is linked to BMI in the hypertensive sib pairs included in this study lies in a region of conserved synteny with a region on rat chromosome 13 found in the F2 cross of Dahl salt-sensitive×Brown Norway rats that contains QTLs for the variability of both diastolic blood pressure on a low salt diet and also for the pressor responsiveness to angiotensin II.49 This rat-human homology provides additional evidence for the validity of the linkage results in the sib pairs and for the relevance of these QTLs to arterial pressure. Further, we have recently reported that introgression of chromosome 13 from Brown Norway rats into the background of Dahl salt-sensitive rats nearly abolishes the salt-induced rise of arterial pressure in the Dahl salt-sensitive rat.50 Although this consomic model does not localize the specific protective regions(s) on chromosome 13, this observation suggests that a powerful gene or set of genes on chromosome 13 of the Brown Norway rat confers protection against the detrimental effect of high salt in the Dahl salt-sensitive rat.

**Perspectives**

Blood pressure is the end result of many interacting control systems, and it is a phenotype that is variable moment to moment. For these reasons, it may be a difficult “phenotypic trait” with which to obtain significant genetic linkage in population studies. Our approach has been to evaluate physiological pathways that are important in the regulation of arterial pressure. The current observations suggest that focusing on the genetic determinants of hypertension-related phenotypes is a promising strategy for identifying the genetic determinants of hypertension. Our long-term strategy is to determine if distinct clusters of hypertension-related pheno-
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