Evidence for a Gene Influencing Blood Pressure on Chromosome 17

Genome Scan Linkage Results for Longitudinal Blood Pressure Phenotypes in Subjects From the Framingham Heart Study


Abstract—Hypertension is a leading cause of morbidity and mortality. Efforts to identify hypertension genes have focused on 3 approaches: mendelian disorders, candidate genes, and genome-wide scans. Thus far, these efforts have not identified genes that contribute substantively to overall blood pressure (BP) variation in the community. A 10-centiMorgan (cM) density genome-wide scan was performed in the largest families from 2 generations of Framingham Heart Study participants. Heritability and linkage for long-term mean systolic and diastolic BP phenotypes were analyzed by use of SOLAR software. Heritability estimates were based on BP measurements in 1593 families. Genotyping was performed on 1702 subjects from 332 large families, and BP data were available for 1585 (93%) genotyped subjects who contributed 12 588 longitudinal BP observations. The mean age was 47 years, and mean BP was 127/80 (systolic/diastolic) mm Hg. Long-term systolic and diastolic BP phenotypes had high heritability estimates, 0.57 and 0.56, respectively. For systolic BP, multipoint log-of-the-odds (LOD) scores >2.0 were located on chromosome 17 at 67 cM (LOD 4.7, \( P = 0.0000016 \)) and 94 cM (LOD 2.2). For diastolic BP, LOD scores >2.0 were identified on chromosome 17 (74 cM, LOD 2.1) and chromosome 18 (7 cM, LOD 2.1). Using a genome-wide scan, we found strong evidence for a BP quantitative trait locus on chromosome 17. Follow-up studies are warranted to identify the gene or genes in this quantitative trait locus that influence BP. Such knowledge could extend our understanding of the genetic basis of essential hypertension and have implications for the evaluation and treatment of patients with high BP. (Hypertension. 2000;36:477-483.)

Key Words: genetics ■ genome scan ■ linkage ■ epidemiology ■ hypertension, essential ■ blood pressure ■ Framingham Heart Study

Hypertension affects about one quarter of adults in industrialized countries, and it contributes to considerable morbidity and mortality from stroke, heart failure, coronary heart disease, and renal failure.1-3 Hypertension is a complex disorder that results from the interplay of genetic and environmental influences. It is hoped that advances in understanding the genetic causes of hypertension will contribute to improvements in the diagnosis and treatment of this common condition. Three approaches to identifying genes for hypertension have been pursued: study of mendelian disorders, candidate gene evaluation, and genome-wide scans. Through the investigation of mendelian disorders affecting blood pressure (BP), several genes have been identified and characterized.4-8 The contribution of these rare conditions to the overall BP variation in the general population, however, is very small.

A second approach to identifying BP/hypertension genes is through the characterization of candidate genes. In recent years, a growing list of candidate genes hypothesized to influence BP has emerged, and for several of these, evidence of linkage or association with hypertension has been reported.9-13 To date, none of these candidate genes, however, has been shown to contribute substantively to the variation in BP in the general population.

The third approach is a genome-wide scan to identify chromosomal regions linked to BP or hypertension. Genome scan results can guide candidate gene research by according greater priority to candidates that are located within areas of...
linkage. Genome scanning may also identify chromosomal regions within which no known candidate genes are recognized; such a finding has the potential to identify novel BP genes. Genome scans are being used in family-based BP studies and have led to reports of multiple linkages\(^1\)\(^2\); however, none of the reported linkages has attained statistical significance at the genome-wide level.

The Framingham Heart Study, which began in 1948, has meticulously characterized BP and other relevant phenotypes in 2 generations of participants. A 10-centiMorgan (cM) density genome-wide scan in subjects from the largest families within the study was recently completed. The repeated measurement of BP in 2 generations of study participants has permitted the characterization of unique, longitudinal BP phenotypes unavailable in other prospective, population-based studies. In contrast to BP studies that selectively recruited hypertensive subjects, Framingham Heart Study subjects were recruited without regard to their BP. Consequently, this study is able to assess linkage across the entire range of BP values. This feature of the study may enhance the detection of linkage, because genes that affect BP may contribute not only to hypertension but also to intermediate and low BP.\(^1\)\(^2\) In addition, our project began in an era when genome scanning may also identify chromosomal regions within which no known candidate genes are recognized; such a finding has the potential to identify novel BP genes. To calculate residuals, the following approach was taken. Let \(y_\text{i}\) denote either systolic or diastolic BP for the \(j\)th examination on the \(i\)th participant, and let \(x_\text{i}\) denote age and \(x_\text{b}\) body mass index; also, let \(y_\text{i}\) denote the subject’s mean BP, and let \(m_1\) and \(m_2\) be his or her mean age and mean body mass index in the stated age range. Sample-wide regressions were used to model BP as a linear function of the subject’s mean age and mean body mass index. Specifically, the model can be stated as \(y_\text{i} = \beta_0 + \beta_1(x_\text{i} - m_1) + \beta_2(m_2 - m_2) + R\), where \(m_1\) and \(m_2\) are sample means for age and body mass index. The residual \(R\) was used for the longitudinal phenotypes.

Systolic and diastolic BP phenotypes were analyzed independently. For diastolic BP, we analyzed data only for ages 25 to 54 years, because diastolic BP declines with advancing age, beginning around age 55 years.\(^1\)\(^9\) Also, regressions were conducted separately for each sex and cohort to accommodate sex and cohort effects.

**Methods**

**Study Subjects**

The Framingham Heart Study began in 1948 with the recruitment of 5209 men and women from Framingham, Mass, who were between 28 and 62 years of age.\(^1\)\(^7\) Subjects underwent a medical history, physician-administered physical examination, laboratory tests, and electrocardiography. Examinations were repeated every 2 years. Beginning in 1971, 5124 offspring and spouses of offspring of original participants were recruited for similar examinations.\(^1\)\(^8\) The offspring cohort was reexamined 8, 12, 16, 20, and 24 years after their initial visit. At each clinic visit, the examining physician measured the seated BP twice with a mercury column sphygmomanometer. The systolic and diastolic BPs were determined by the first and fifth Korotkoff sounds, respectively. Two BP measurements were averaged to derive the systolic and diastolic pressures for that examination. Body mass index was calculated as the weight in kilograms divided by the square of height in meters (kg/m\(^2\)). All subjects gave informed consent before each clinic visit, and the examination protocol was approved by the Institutional Review Board at Boston Medical Center (Boston, Mass).

**BP Phenotypes**

**Longitudinal BP Phenotypes**

All BP measurements taken when subjects were aged 25 to 75 years were analyzed. The following criteria were stipulated: (1) there had to be at least 10 years between a subject’s initial and final examinations within the age range; (2) at least 4 examinations within the age range were required for the original cohort and at least 3 for offspring cohort participants; and (3) height and weight measurements were required, but if weight was missing, the most recent measurement within 4 years was used.

Longitudinal BP data were analyzed for 8478 subjects who met these criteria by using a 2-stage procedure: (1) within-subject mean BP was calculated and (2) sample-wide regressions were used to adjust for age and body mass index, yielding a residual for each subject. These residuals constitute the longitudinal BP phenotypes.

**Genotyping**

DNA was extracted from whole-blood or buffy coat specimens by using a standard protocol.\(^2\)\(^1\)\(^2\) DNA aliquots from subjects within the largest Framingham Heart Study families were sent in 4 batches to the Mammalian Genotyping Service Laboratory at the Marshfield Clinic (Marshfield, Wis), where a 10-cM density genome scan was performed (marker set 8A, average heterozygosity 0.77). (Details regarding markers and primers are available on the World Wide Web at [http://www.mshrd.umn.edu/genetics/default.html](http://www.mshrd.umn.edu/genetics/default.html).) Genotype data cleaning consisted of 2 steps: Family relationships were verified on the basis of all available markers by using the sib_kin program of the ASPEX ([ftp://ahmed.stanford.edu/pub/aspey/index.html](ftp://ahmed.stanford.edu/pub/aspey/index.html)) package.
Mendelian inconsistencies were detected and eliminated by using the GENTEST program (http://www.sfbr.org/sfbr/public/software/software.html).

Heritability and Linkage Analysis

Heritability estimates were obtained by using variance-component methodology implemented in the SOLAR package and were based on phenotype data from 1593 families for systolic BP and 1300 families for diastolic BP. Two-point and multipoint quantitative trait linkage analyses were conducted on the standardized residuals for longitudinal systolic and diastolic BPs by using the SOLAR package. In this approach, genotypes are imputed for untyped individuals, conditional on all other marker data and pedigree structure, and the proportion of marker alleles shared identical by descent among all relative pairs is estimated. Therefore, individuals who are not genotyped but have phenotype data contribute to the linkage results. The pedigree-based approach of SOLAR is more powerful than sib-pair analysis when data on extended families are available. Linkage is assessed by fitting a polygenic model that does not incorporate genetic marker information (ie, identical by descent status) and comparing it with models that incorporate genotype data at a specific marker (2-point analysis) or across a chromosome (multipoint analysis). The log (base 10) of the ratio of the likelihoods of the polygenic and marker-specific models is the log-of-the-odds (LOD) score, the traditional measure of genetic linkage.

Results

Study Sample

There were a total of 1702 genotyped individuals from 332 extended families, with family sizes ranging from 2 to 29 genotyped individuals. The genotyped sample included the following relative pairs: parent-offspring pairs, n=933; sibling pairs, n=1545; cousin pairs, n=742; and avuncular pairs, n=468. Criteria for analysis of longitudinal BP phenotypes were met in 2498 (1585 genotyped) subjects for systolic and 1925 (1294 genotyped) subjects for diastolic BP. The genotyped sample included 393 members of the original cohort and 1192 of the offspring cohort; 47% were men. Characteristics of the genotyped study sample are shown in Table 1. The genotyped study subjects contributed 12 588 longitudinal (repeated) BP measurements (an average of 7.9 per subject; range, 3 to 20) spanning up to 38 years for original cohort subjects and 20 years for offspring subjects. Mean age was 47 years, and mean body mass index was 26.4 kg/m². Subjects were hypertensive (systolic BP 140 mm Hg or higher, diastolic BP 90 mm Hg or higher, or current drug treatment for high BP) at 27.9% of visits, but only 11.3% of genotyped BP observations reflected treatment with antihypertensive medication. The average BP (systolic/diastolic) was 124/78 mm Hg (unadjusted) versus 127/80 mm Hg after adjustment for hypertension treatment. Departure from normality of BP residuals was minimal, as measured by skewness (0.70 for systolic BP and 0.67 for diastolic BP) and kurtosis (0.93 for systolic BP and 0.91 for diastolic BP). Long-term systolic and diastolic BP phenotypes had high heritability estimates, 0.57 and 0.56, respectively. The corresponding heritability estimates for single-examination BPs were 0.42 and 0.39.

Two-Point and Multipoint Linkage

The highest 2-point LOD scores for long-term systolic and diastolic BP traits for each of the 22 autosomes are presented in Figure 1. For systolic BP, 2-point LOD scores of 2.0 or higher were observed for markers on chromosomes 5 and 10 and for 2 markers on chromosome 17 (Table 2). For diastolic

---

TABLE 1. Characteristics of the 1585 Genotyped and Phenotyped Subjects: Values From Longitudinal Observations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean or Percent</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of examinations</td>
<td>12 588</td>
<td></td>
</tr>
<tr>
<td>No. of examinations per subject</td>
<td>7.9</td>
<td>6</td>
</tr>
<tr>
<td>Men, %</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>47.5</td>
<td>8.8</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Systolic BP, mm Hg (unadjusted)</td>
<td>124.3</td>
<td>14.3</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg (unadjusted)</td>
<td>78.2</td>
<td>7.9</td>
</tr>
<tr>
<td>Systolic BP, mm Hg (adjusted)</td>
<td>126.8</td>
<td>17.4</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg (adjusted)</td>
<td>79.5</td>
<td>9.1</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>27.9</td>
<td></td>
</tr>
<tr>
<td>Hypertension treatment, %</td>
<td>11.3</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Maximum 2-point LOD scores for long-term systolic (open bars, SBP) and diastolic (closed bars, DBP) BP phenotypes for the 22 autosomes.
BP, the only 2-point LOD score of 2.0 or higher was on chromosome 9; a LOD score of 1.6 was found on chromosome 17 (marker GATA25A04 [D17S1299]) and a LOD score of 1.9 for chromosome 18 (AFM321xc9 [D18S481]).

Figure 2 depicts the highest multipoint LOD scores for each of the autosomes. For systolic BP, multipoint LOD scores of 2.0 or higher were found on chromosome 17 at 2 regions (67 cM, LOD 4.7; and 94 cM, LOD 2.2; Figure 3), and an additional region of interest was identified on chromosome 5, with a multipoint LOD score of 1.9 at 23 cM. For diastolic BP, LOD scores of 2.1 were located on chromosome 17 at 74 cM (Figure 3) and on chromosome 18 at 7 cM (Figure 4). LOD scores for 2 locations that were suggestive in 2-point analyses (chromosome 10 for systolic BP and chromosome 9 for diastolic BP) diminished in the multipoint analyses (LOD scores of 0.72 and 1.2, respectively); examination of the 2-point data revealed that in each case, flanking markers showed little or no evidence of linkage (LOD scores ranged from 0.0 to 0.23), thereby reducing the magnitude of the LOD scores at these locations in the multipoint analysis.

Discussion

Using a 10-cM density genome-wide scan in participants from the Framingham Heart Study, we found significant evidence of linkage of longitudinal systolic BP to chromosome 17q12–21, with a LOD score of 4.7, with odds of 50,000:1 in favor of linkage (corresponding \( P \) value of 0.0000016). To our knowledge, this is the first population-based study to report linkage of BP that attained significance at a genome-wide level.24

These results provide overwhelming evidence for a BP quantitative trait locus (QTL) in this interval. First, the LOD score substantially exceeds the minimum threshold for significant linkage in such a genome-wide study. Second, this interval has been implicated in hypertension.25,26 Third, there are plausible candidate genes in the interval. Fourth, the highest LOD score for diastolic BP lies in the same interval, providing corroborating evidence. Finally, this interval is homologous with BP QTLs in the rat and mouse.

A major challenge confronting the field of hypertension genetics is the need for better BP phenotypes.27 The Framingham Heart Study is unique among prospective epidemiological studies by virtue of its longevity and multigenerational structure. The serial measurement of BP in study participants facilitated the characterization of longitudinal BP phenotypes that are unavailable in newly recruited family studies. Specifically, BPs obtained in the same age interval were available for 2 generations of participants. Moreover, because the overwhelming majority of BP measurements were obtained in untreated subjects, we were able to study BP as a quantitative trait, in contrast to many current studies, which are limited to classification of qualitative phenotypes. Finally, this is 1 of a small number of large studies performing a comprehensive genome-wide analysis of linkage, rather than simply testing candidate genes or chromosome intervals, thereby permitting an unbiased search for BP QTLs. As a result, this comprehensive linkage study offers a unique and powerful approach to the detection of BP QTLs in the general population.

The LOD-1 interval (the support interval for which the LOD score equals the maximum minus 1) of the location of the chromosome 17 QTL spans a 16-cM interval at 17q12–21, flanked by loci GGAA7D11 (D17S1293) and GATA49C09 (D17S1290). This interval is of great interest,
as it has been previously implicated in BP variation in humans. Pseudohypoaldosteronism type II, an autosomal dominant form of hypertension with hyperkalemia, is linked to this interval.\textsuperscript{28} Moreover, 2 other studies have provided evidence of increased allele sharing among hypertensive siblings at this location, although neither of these studies reached the threshold for significance in a genome-wide analysis.\textsuperscript{25,26} This QTL is also of particular interest, because it is syntenic with a QTL on rat chromosome 10 that was reported to be linked to hypertension in several studies of spontaneously hypertensive rats.\textsuperscript{29–32} Furthermore, in a salt-sensitive hypertensive mouse strain, Paigen et al\textsuperscript{33} found linkage to BP on mouse chromosome 11 that is homologous with rat chromosome 10q and human chromosome 17q, indicating a remarkable concordance across 3 species.

Examination of databases reveals many known genes and expressed sequence tags in or near the 16-cM QTL interval at 17q12–21. No genes that have been strongly implicated in BP variation lie in this interval; however, potential candidate genes in the interval include the $\alpha$-1 thyroid receptors, the neuronal homologue of the amiloride-sensitive epithelial sodium channel, the corticotropin-releasing hormone receptor 1, insulin-like growth factor–binding protein-4, hepatocyte nuclear factor 1-\(\beta\), and the chloride/bicarbonate exchanger AE1. It also is possible that the gene underlying the QTL locus has not yet been identified.

In addition to the chromosome 17q12–21 interval, there are 2 additional regions yielding LOD scores $\geq 2.0$ in multipoint analyses. One of these lies just distal on chromosome 17, and it is possible that this locus is independent of our largest peak. This second chromosome 17 peak overlaps the locus encoding the angiotensin-converting enzyme locus,\textsuperscript{34} a much-studied candidate gene for hypertension.\textsuperscript{10} The final locus is on chromosome 18, an interval that, to our knowledge, has not been previously implicated in BP variation. An interesting candidate gene in the chromosome 18 interval is the melanocortin receptor 2, which is the physiological receptor for corticotropin. Given the known effects of glucocorticoids on BP, it is of interest that receptors involved in the regulation of cortisol secretion lie in both the chromosome 17 and chro-
mosome 18 intervals. The locations on chromosomes 5, 9, and 10 identified in 2-point analyses remain regions of interest, despite the reduced evidence of linkage in the multipoint analyses. Reasons for differences in LOD scores between 2-point and multipoint results include map or genotyping errors, large gaps between adjacent markers, or isolated false-positive results.

**Study Strengths and Limitations**

The Framingham Heart Study recruited 2 generations of subjects without regard to their BP and followed them up prospectively. BP phenotypes were sex-specific and were adjusted for age and body mass index, the 2 leading determinants of BP in the general population. The high estimates of heritability of our long-term BP phenotypes, 0.57 for systolic and 0.56 for diastolic BP, are considerably higher than those of single-examination systolic and diastolic BP, 0.42 and 0.39, respectively, and are an indication of the far greater value of the long-term BP phenotypes available in the Framingham data.

The study has several potential limitations. First, although we believe that the adjustment of BP for subjects who were receiving antihypertensive drug treatment is a major strength of our study, it is possible that it introduced a degree of misclassification. Of note, evidence for linkage at our most promising regions remained when actual BP measurements were used in place of adjusted values. Second, this study is based on a sample of white subjects. The extent to which our findings apply to other racial or ethnic groups, in whom the prevalence of hypertension differs from that in whites and in whom a different mix of genes may be important in BP regulation, deserves investigation. Third, there are likely to be additional QTLs involved in the expression of BP that we were unable to detect. Specifically, there was ample power to detect a QTL that explains 25% to 30% of the variation in BP. On the basis of the pedigree structure in the current study, we had 75% to 80% power to detect LOD scores >3 for a QTL that explains 25% of the variation, and >90% power to detect a QTL that explains 30% of the variation, but <50% power to detect an LOD score >3 for a QTL that explains 20% of the variation. To detect an LOD score >2, we had ~70% to 75% power for a QTL that explains 20% of the variation but <50% power for a QTL that explains only 15% of the variation. Fourth, it is possible that some of the positive linkage findings represent false results. Based on the pedigree structure of the 332 families used for linkage in this investigation, simulation studies indicate that in the absence of linkage, the mean number of 2-point LOD scores >2 that can be expected for this genome scan is 0.5. We observed LOD scores >2 for 4 markers for systolic BP and for 1 marker for diastolic BP, several more than would be expected by chance.

**Future Directions**

Progress in identification of the chromosome 17q QTL can progress on several fronts. Success in positional cloning of the genes underlying pseudohypoaldosteronism type II and the sympathetic rodent QTL has the potential to identify the gene underlying this BP QTL. The location of the QTL itself can potentially be refined by genotyping additional markers and analyzing linkage in the critical interval. Moreover, genes in the interval can be screened for variants, and these can be examined for linkage disequilibrium with BP. Evaluation of the functional consequences of identified variants in vitro or in animal models can strengthen evidence for putative functional variants.

Ultimately, the genes contributing to BP variance will be pinpointed from BP QTLs, and their functional mutations will be identified. The completion of a draft version of the human genome sequence will identify and precisely locate roughly 90% of all human genes, greatly augmenting efforts to identify QTLs underlying human disease. Our finding of at least 1 BP QTL in the general population increases the likelihood that identification of the underlying gene or genes may prove clinically important for the evaluation and treatment of patients with hypertension.

**Acknowledgments**

The Framingham Heart Study is supported by the National Heart, Lung, and Blood Institute (NHLBI, National Institutes of Health), Bethesda, Md, through contract N01-HC-38038. Portions of this work were supported by National Institutes of Health/US Public Health Service SCOR in Hypertension grants P50HL55001 (to A.L.D. and H.G.) and P50HL55007 (to R.P.L.). R.P.L. is an investigator of the Howard Hughes Medical Institute. The authors would like to acknowledge the contributions of Drs Ernst J. Schaefer and Jose M. Ordovas, who isolated the DNA that made this study possible, with funding from NHLBI grant HL54776 (to J.M.O.).

**References**


25. Levy et al Genetic Influence of BP in Framingham Heart Study Subjects


Evidence for a Gene Influencing Blood Pressure on Chromosome 17: Genome Scan Linkage Results for Longitudinal Blood Pressure Phenotypes in Subjects From the Framingham Heart Study


*Hypertension*. 2000;36:477-483
doi: 10.1161/01.HYP.36.4.477

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/36/4/477

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Hypertension* 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 *Hypertension* is online at:
http://hyper.ahajournals.org//subscriptions/