Genetic analysis of blood pressure in C3H/HeJ and SWR/J mice

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DiPetrillo, Keith, Shirng-Wern Tsaih, Susan Sheehan, Conrado Johns, Peter Kelmenson, Haralambos Gavras, Gary A. Churchill, and Beverly Paigen. Genetic analysis of blood pressure in C3H/HeJ and SWR/J mice. Physiol Genomics 17: 215–220, 2004. First published March 2, 2004; 10.1152/physiolgenomics.00212.2003.—Hypertension is a complex phenotype induced by multiple environmental and genetic factors. Quantitative trait locus (QTL) analysis is a powerful method for identifying genomic regions underlying complex diseases. We conducted a QTL analysis of blood pressure in mice using 217 F2 progeny (males and females) from a cross between the normotensive C3H/HeJ and hypertensive SWR/J inbred strains. Our analysis identified significant QTL controlling blood pressure on chromosome 1 [Chr 1; Bpq8: peak 78 cM; 95% confidence interval 64–106 cM; logarithm of the odds ratio (LOD) 3.5; peak marker D1Mit105] and on Chr 16 (Bpq9; peak 56 cM; 95% confidence interval 46–58 cM; LOD 3.6; peak marker D16Mit158). Bpq8 was previously identified in a cross between C57BL/6J and A/J mice, and we narrowed this QTL from 42 to 18 cM (95% confidence interval 68–86 cM) by combining the data from these crosses. By examining Bpq8 for regions where ancestral alleles were conserved among the high allele strains (C57BL/6J, SWR/J) and different from the low allele strains (A/J, C3H/HeJ), we identified a 2.3-cM region where the high allele strains shared a common haplotype. Bpq8 is concordant with known QTL in both rat and human, suggesting that the causal gene underlying Bpq8 may be conserved as a disease gene in human hypertension.

METHODS

Mice. C3H and SWR mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and mated to obtain reciprocal F1 populations (C3H×SWR; SWR×C3H). The F2 mice were intercrossed to produce 217 F2 mice (102 female, 115 male). All mice were maintained on a 14:10-h light/dark cycle, housed in cages containing pine shaving bedding and topped with a polyester filter, and allowed access to acidified water and food (18% protein rodent diet, product 2018; Harlan Teklad, Madison, WI) ad libitum. All animal protocols were reviewed and approved by the Animal Care and Use Committees at The Jackson Laboratory and Boston University.

Phenotyping. The BP-2000 blood pressure analysis system (Visitech Systems, Apex, NC) was used to measure tail-cuff blood pressure and heart rate in the mice as previously described (10, 11). Four mice were placed on the warming platform (37°C) equipped with restrainers. Each tail was placed into a computer-controlled tail cuff, and a photoresistor cell below each tail detected pulse. To improve data accuracy, blood pressure was measured in 8-wk-old mice, all measurements were taken in the morning, and mice were trained to the machine and the measurement procedure for 5 days before data were collected. Following the training period, data were collected for the next 5 days. Mice were allowed to acclimate to the tail-cuff blood pressure measurement during five preliminary blood pressure cycles. After the preliminary cycles, blood pressure and heart rate of each mouse were measured 20 times daily.

To minimize the impact of measurement anomalies on our analysis, we did not analyze: 1) readings below 60 mmHg (assumed to result from failure to detect the pulse of a moving mouse); 2) readings of more than two standard deviations from the mean for an individual mouse on a given day; 3) data from any mouse on days when we obtained less than eight successful readings; and 4) data from any mouse with less than 3 days of satisfactory data. Satisfactory data were averaged to yield a single blood pressure reading for each mouse. Thus every blood pressure value used in our analysis was based on a minimum of 24 and a maximum of 100 readings per review, see Korstanje and Paigen, Ref. 5). The relevance of QTL analysis in rodents is supported by the observation that many QTL found in rodents are concordant to known hypertension QTL in humans (9, 10). This enables the use of inbred animal models to identify and narrow QTL more efficiently than in humans, with reasonable expectation that the findings will be applicable to human hypertension.

In the present study, we performed a QTL analysis of male and female progeny from an intercross between hypertensive SWR/J (SWR) and normotensive C3H/HeJ (C3H) mice to identify the following: 1) independent and interactive QTL that modulate blood pressure and heart rate; and 2) sex differences in the genomic loci regulating blood pressure. We confirmed the existence of two previously identified QTL, and we narrowed one of these QTL by using a novel statistical method to combine the data from multiple crosses and through haplotype analysis.

HYPERTENSION AFFECTS 20% of Americans and substantially contributes to cardiovascular and renal disease in the United States (1). Hypertension is present in about 50% of patients with first myocardial infarction and 66% of patients with first stroke (1). Hypertension is associated with a two- to threefold increased risk of congestive heart failure and precedes development of the disease in 91% of patients (6). In addition, hypertension is a major cause of end-stage renal disease (2, 4). Considering the detrimental impact of hypertension on cardiovascular and renal health, improved understanding of the causes of hypertension will likely enhance prevention and treatment of the disease.

The etiology of a disease can be partly understood by identifying the genetic factors that contribute to its onset or severity. Quantitative trait locus (QTL) analysis is a powerful technique for uncovering genomic regions that influence disease, a first step toward identifying specific causal genes. In fact, QTL analysis in model organisms has been successfully employed to identify genes underlying various diseases (for
mouse. We also used criteria 2–4 to eliminate unsatisfactory heart rate measurements.

Genotyping. Genomic DNA was isolated from the tail of each mouse and genotyped with 103 simple-sequence length polymorphism (SSLP) markers spaced throughout the genome. Primer pairs were purchased from Research Genetics (Huntsville, AL). Fluorescent primers were used to amplify SSLP regions, and PCR products were analyzed using an ABI 3700 capillary electrophoresis analyzer. Markers displaying aberrant, missing, or questionable genotypes were repeated.

QTL analyses. Blood pressure and heart rate QTL were identified with Pseudomarker software using a previously published three-step analysis of genome-wide scans (8, 11). First, main QTL associated with blood pressure and heart rate were detected by computing a logarithm of the odds ratio (LOD) score at 2-cM steps over the genome. The resulting LOD scores were compared with significance thresholds (genome-wide adjusted P = 0.10, suggestive; P = 0.05, significant) computed by permutation analysis (3). Confidence intervals were calculated by the method of Sen and Churchill (8) by finding the region under the posterior density curve containing 95% of the total area. Second, a simultaneous search for pairs analysis was used to detect interacting QTL (8). Finally, all of the QTL were integrated into a multiple regression model to assess their combined effects on blood pressure and heart rate. P values were calculated from the tabulated F distribution using Matlab software.

Statistically combining crosses. We combined the raw data for chromosome 1 (Chr 1) from the cross between C3H and SWR with that from the cross using B6 and A mice by recoding B6 and SWR genotypes as a single high blood pressure allele and A and C3H genotypes as a single low blood pressure allele. A LOD score was computed at 2-cM intervals across the QTL interval for each cross separately and then for both crosses combined. The combined data was analyzed with sex and cross as additive covariates.

Statistical analysis. Values are given as means ± SE. Blood pressure, heart rate, and gene expression values between parental strains and/or F2 offspring were compared using ANOVA followed by the Student-Newman-Keuls posttest. P < 0.05 was considered significant.

RESULTS AND DISCUSSION

Blood pressure in parental strains and F2 progeny. We utilized the SWR and C3H inbred mouse strains because their baseline blood pressures were significantly different (Table 1). Blood pressures of SWR mice were ~15 mmHg higher than those of C3H mice. Sex differences within the parental strains were not statistically significant (Table 1). Mean blood pressures of F2 males and females were intermediate to the parental strains (Table 1). Although sex differences in blood pressure were absent in the parental strains, F2 females exhibited a significant elevation in blood pressure compared with F2 males (Table 1).

Identification of blood pressure QTL. All F2 mice were individually genotyped for 103 SSLP markers spaced throughout the genome, enabling us to detect both additive and interacting QTL that affect blood pressure. The F2 population displayed a normal blood pressure distribution (Fig. 1). The

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**Genetic Analysis of Blood Pressure: C3H/HJ and SWR/J Mice**

**Table 1. Blood pressure and heart rate in C3H, SWR, and (C3H×SWR) F2 mice**

<table>
<thead>
<tr>
<th></th>
<th>C3H</th>
<th>(C3H×SWR) F2</th>
<th>SWR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>105±2</td>
<td>111±1†‡</td>
<td>119±2*</td>
</tr>
<tr>
<td>Females</td>
<td>108±3</td>
<td>117±1†‡</td>
<td>126±3*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>585±7‡</td>
<td>661±4†‡</td>
<td>635±8‡</td>
</tr>
<tr>
<td>Females</td>
<td>623±9</td>
<td>667±4*</td>
<td>692±11*</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 10 (C3H and SWR, each sex), n = 115 (F2 males), and n = 102 (F2 females). Blood pressure and heart rate were measured using an automated tail-cuff as described in METHODS. †P < 0.05 vs. C3H, same sex. ‡P < 0.05 vs. SWR, same sex. †‡P < 0.05 vs. opposite sex, same strain.

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**Fig. 1. Distribution of blood pressure in the F2 progeny.**

**Fig. 2. Genome-wide scan for blood pressure and heart rate quantitative trait loci (QTL).** Genome-wide scan for QTL underlying blood pressure (A) and heart rate (B). Suggestive (P = 0.10) and significant (P = 0.05) logarithm of the odds ratio (LOD) scores, as determined by permutation analysis, are shown as dotted lines.
The QTL detected on Chr 1, Bpq8, was broad (Fig. 3A) and overlapped a blood pressure QTL (Bpq2; 70–90 cM) previously identified in a cross between C57BL/6J (B6) and A/J (A) mice (10). The SWR Bpq8 allele was dominant, since mice carrying one copy of the SWR allele at Bpq8 had blood pressure equivalent to SWR homozygotes and significantly higher than C3H homozygotes (Fig. 3B). The Chr 16 QTL (Fig. 3C), which we named Bpq9, had a smaller 95% confidence interval than Bpq8. Bpq9 also overlapped a previously identified blood pressure QTL found in a cross between BPH/2 and BPL/1 (13). Its allelic effects were more complicated than those for Bpq8, homozygotes for either the C3H or SWR allele at this locus had significantly higher blood pressure than heterozygotes (Fig. 3D).

The effect of sex on blood pressure explained 8.5% of the variation in F2 progeny, based on multiple linear regression analysis (Table 3). Bpq8 and Bpq9, respectively, accounted for 4.5% and 5.9% of the F2 population variance. Neither of the QTL was sex dependent (data not shown), nor did we detect any other sex-specific effects by scanning the genome for interaction between QTL and sex. Thus it appears that the same genetic factors influenced blood pressure in both sexes in this F2 population. Additional experiments will be required to characterize the observed sex difference in blood pressure.

Evidence for two QTL on Chr 16. The allele effect plot for Bpq9 suggests a complex interaction of alleles. We hypothesized that this QTL might actually be two closely linked QTL with opposite allelic effects. We tested this hypothesis by fitting a multiple regression model for both one and two QTL at this locus and found that LOD score for the two-QTL model (6.97) was higher than for the one-QTL model (3.6). A change in LOD of ≥3 is significant for the presence of two QTL at this locus. Additionally, the final multiple regression model suggested that these two QTL (at 44 and 54 cM) may have interactive effects on blood pressure (Table 4). Unfortunately, we were not able to discern the allelic effects at both positions because of their proximity.

Combining crosses to narrow Bpq8. Both blood pressure QTL detected in this analysis, Bpq8 and Bpq9, have been previously identified in other QTL analyses (10, 13). Finding repetitive QTL in crosses with different strains implies that only a few polymorphic genetic loci regulate blood pressure in the genome-wide scan indicated significant blood pressure QTL on Chrs 1 and 16 (Fig. 2A; the chromosomal locations, peak markers, confidence intervals, and LOD scores are presented in Table 2). We found neither suggestive nor significant QTL for heart rate (Fig. 2B) and detected no significant interacting QTL in this analysis.

The LOD, logarithm of the odds ratio; QTL, quantitative trait locus; Chr, chromosome; CI, confidence interval; The LOD threshold, determined by permutation testing, was 2.6 for suggestive QTL (P = 0.10) and 3.0 for significant QTL (P = 0.05). *Data from overlapping Bpq8 and Bpq2 (C57BL/6J, high allele; A/J, low allele) were combined to narrow the QTL interval.

Table 2. Chromosomal locations, peak markers, confidence intervals, and LOD scores for blood pressure QTL

<table>
<thead>
<tr>
<th>Name</th>
<th>Chr</th>
<th>Peak, cM</th>
<th>95% CI, cM</th>
<th>High Allele</th>
<th>Inheritance</th>
<th>Peak Marker</th>
<th>LOD</th>
<th>Previous Reports</th>
</tr>
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<tr>
<td>Bpq8</td>
<td>1</td>
<td>78</td>
<td>64–106</td>
<td>SWR</td>
<td>Dominant</td>
<td>D1Mit105</td>
<td>3.5</td>
<td>Ref. 10</td>
</tr>
<tr>
<td>Bpq8/Bpq2*</td>
<td>1</td>
<td>74</td>
<td>68–86</td>
<td>C3H/SWR</td>
<td></td>
<td>D16Mit158</td>
<td>3.6</td>
<td>Ref. 13</td>
</tr>
</tbody>
</table>

LOD, logarithm of the odds ratio; QTL, quantitative trait locus; Chr, chromosome; CI, confidence interval; The LOD threshold, determined by permutation testing, was 2.6 for suggestive QTL (P = 0.10) and 3.0 for significant QTL (P = 0.05). *Data from overlapping Bpq8 and Bpq2 (C57BL/6J, high allele; A/J, low allele) were combined to narrow the QTL interval.

Fig. 3. Fine mapping and allelic effects of blood pressure QTL. Fine mapping plots are shown for Bpq8 on Chr 1 (A) and Bpq9 on Chr 16 (C) identified in the genome scan. The effects of the C3H ("C") and SWR ("S") alleles at Bpq8 (B) and Bpq9 (D) on blood pressure are also displayed. Values from male and female F2 offspring were combined since the QTL were independent of sex. Blood pressure values are expressed as means ± SE. PPD, posterior probability density of QTL location. *P < 0.05 vs. C/C genotype. *P < 0.05 vs. S/S genotype.
inbred mice and that these repetitive QTL may have arisen from conserved ancestral alleles. A novel method for narrowing repetitive QTL is combining data from multiple crosses (Li R, Lyons MA, Wittenburg H, Paigen B, and Churchill GA, unpublished observations). Since QTL localization is dependent on the locations of recombination breakpoints, combining crosses can effectively narrow the confidence interval by providing more recombinations within the region. We combined the raw data for Bpq8 with that for Bpq2 by recoding B6 and SWR genotypes as a single high blood pressure allele and A and C3H genotypes as a single low blood pressure allele. We then reanalyzed the combined Chr 1 data for linkage with blood pressure. Combining the crosses reduced the confidence interval from 42 to 18 cM, spanning 68–86 cM on Chr 1 (Fig. 4). Additionally, the LOD score for Bpq8 increased to 6.38 (Table 2).

**Haplotype analysis of Bpq8.** Recent evidence demonstrates that inbred mouse strains are genetic mosaics of alleles principally derived from *Mus musculus musculus* and *M. m. domesticus* ancestors (12). Wade and colleagues (12) suggest that QTL can be effectively narrowed by comparing genomic sequences of the parental strains across the region, especially if the QTL is evident in multiple crosses using different strains. One method of genomic comparison is haplotype analysis. Thus we compared haplotypes from all four parental strains throughout the overlapping QTL interval to identify genomic regions of common ancestral origin between the strains carrying the high blood pressure alleles (B6 and SWR) that differed from the low allele strains (A and C3H). We genotyped 156 SSLP markers over the 65.5 Mb QTL region, which provided an average of 1 marker per 0.42 Mb. We considered a common haplotype to be three or more consecutive shared alleles, although this strategy will miss small haplotypes that may be evident with more densely spaced markers. Using this strategy, we identified a 1.8-Mb interval between D1Mit397 to D1Mit425 and a 2.5-Mb region between D1Mit219 and D1Mit504 where B6 and SWR shared ancestral alleles that differed from A and C3H mice (Fig. 5).

**Table 3. Multiple regression analysis for blood pressure**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Variance Explained</th>
<th>F Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1</td>
<td>1,775</td>
<td>8.5%</td>
<td>22.8</td>
<td>0.001</td>
</tr>
<tr>
<td>D1Mit105</td>
<td>2</td>
<td>943</td>
<td>4.5%</td>
<td>6.0</td>
<td>0.01</td>
</tr>
<tr>
<td>D16Mit158</td>
<td>2</td>
<td>1,226</td>
<td>5.9%</td>
<td>7.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Total</td>
<td>216</td>
<td>20,852</td>
<td>21.2%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DF, degrees of freedom; SS, sums of squares.

**Table 4. Evidence for two, interactive QTL on chromosome 16**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type III SS</th>
<th>Variance Explained</th>
<th>F Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr 1 (80 cM)</td>
<td>2</td>
<td>944</td>
<td>4.5%</td>
<td>6.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Chr 16 (44 cM)*</td>
<td>6</td>
<td>1,190</td>
<td>5.7%</td>
<td>2.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Chr 16 (54 cM)*</td>
<td>6</td>
<td>1,523</td>
<td>7.3%</td>
<td>3.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Chr 16 (44 cM):Chr16 (54 cM)†</td>
<td>4</td>
<td>1,142</td>
<td>5.5%</td>
<td>3.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Total</td>
<td>216</td>
<td>20,852</td>
<td>27.1%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Variance explained includes both main and interaction effect (hence, 6 DF). †Variance explained is only the interaction effect (hence, 4 DF).

The use of combined data from multiple crosses and haplotype analysis to narrow the initial QTL interval illustrates the power of finding repetitive QTL. Statistically combining the crosses did not require additional laboratory experiments but simply utilized existing data more effectively. In the end, combining the crosses reduced the confidence interval by 60% (from 42 to 18 cM) and substantially decreased the number of potential candidate genes. Haplotype analysis of this QTL further narrowed the interval to ~2.3 cM and required only additional genotyping and no additional mice. Overall, combining crosses and haplotype analysis substantially focused our search for candidate genes from the initial 42-cM QTL confidence interval to 2.3-cM region with minimal laboratory work beyond the initial crosses.

**Comparative mapping.** Comparative genomics presents an opportunity for QTL narrowing when the QTL is conserved across species. By comparing the homology of a QTL region across species, the ends of a QTL region in one species can delimit the concordant QTL in another species. Bpq8 is homologous to rat Chr 13 and human Chr 1, which each contain blood pressure QTL (7, 14). Based on comparative map positions from Ensembl, the rat and human QTL both map to roughly 68–102 cM on mouse Chr 1. This spans the narrowed Bpq8 confidence interval of 68–86 cM and does not further narrow the QTL. However, finding concordant blood pressure QTL in rat and human implies that the causal gene underlying Bpq8 may be a conserved disease gene in these species.

In summary, by performing a QTL analysis of a (C3H × SWR) F2 population, we confirmed the existence of two QTL regulating blood pressure: Bpq8 on Chr 1 and Bpq9 on Chr 16. Finding repetitive QTL in different crosses facilitates the use of comparative genomic methods, such as statistical methods to combine crosses (Li et al., unpublished observations), haplotype analysis (12), and cross-species comparisons (10), to identify the underlying genetic polymorphisms. Bpq8 repre-
sents an excellent candidate QTL for these methods, because it overlaps \(Bpq2\) (found between B6 and A mice) and is concordant with known hypertension QTL in both rat and human. We effectively narrowed \(Bpq8\) by combining the data with those for \(Bpq2\), and we identified a 2.3-cM interval where the high allele strains share a common haplotype. Understanding the genetic basis for \(Bpq8\) and other hypertension QTL may provide important insight into the pathophysiology of hypertension.

ACKNOWLEDGMENTS

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GRANTS

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