Physiol Genomics 10: 5–12, 2002.
First published April 30, 2002; 10.1152/physiolgenomics.00002.2002.

QTL associated with blood pressure, heart rate, and heart weight in CBA/CaJ and BALB/cJ mice

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Received 7 January 2002; accepted in final form 29 April 2002

Sugiyama, Fumihiro, Gary A. Churchill, Renhua Li, Laura J. M. Libby, Tonya Carver, Ken-Ichi Yagami, Simon W. M. John, and Beverly Paigen. QTL associated with blood pressure, heart rate, and heart weight in CBA/CaJ and BALB/cJ mice. *Physiol Genomics* 10: 5–12, 2002. First published April 30, 2002; 10.1152/physiolgenomics.00002.2002.—To better understand the genetic basis of essential hypertension, we conducted a quantitative trait locus (QTL) analysis of a population of 207 (BALB/cJ × CBA/CaJ) F_2 male mice to identify genomic regions that regulate blood pressure, heart rate, and heart weight. We identified two loci, *Bpq6* (blood pressure quantitative locus 6) on chromosome 15 (Chr 15; peak, 16 cM; 95% confidence interval, 0–25 cM) and *Bpq7* on Chr 7 (peak, 42 cM; 95% confidence interval, 35–50 cM) that were significantly associated with blood pressure. We also identified two loci, *Hrq1* (heart rate quantitative locus 1) and *Hrq2*, on *D2Mit304* (peak, 72 cM; 95% confidence interval 60–80 cM) and *D15Mit184* (peak, 25 cM; 95% confidence interval 20–35 cM), respectively, that were significantly associated with heart rate. A significant gene-gene interaction for heart rate was found between *Hrq1* and *D1Mit10* (peak, 57 cM; 95% confidence interval, 45–75 cM); the latter QTL was named *Hrq3*. We identified a significant locus for heart weight, *Hwq1* (heart weight quantitative locus 1), at *D14Mit67* (peak, 38 cM; 95% confidence interval, 20–43 cM). Identification of the genes for these QTL should lead to a better understanding of the causes of essential hypertension.

quantitative trait loci; hypertension

HUMAN HYPERTENSION greatly increases the risk of coronary heart disease, congestive heart failure, stroke, and kidney disease. Additionally, it is often associated with and complicated by obesity, dyslipidemia, and non-insulin-dependent diabetes mellitus (1). More than 50 million Americans suffer from hypertension, and 12.7 million of them alleviate its symptoms by taking hypertensive drugs (27). Although human hypertension exists in a monogenic form, most humans suffer from polygenic or essential hypertension. It is difficult to identify the genes that cause essential hypertension in humans, because the genetic backgrounds and environmental influences are almost impossible to control. On the other hand, both genetic background and environment are relatively easy to control in inbred rodent populations. As a result, multiple studies of hypertension in genetic crosses of inbred strains have identified many quantitative trait loci (QTL) associated with blood pressure in the rat (18, 28) and in the mouse (29, 33). In addition, numerous studies of genetically engineered mouse strains with deficiency or overexpression of proteins thought to be involved in blood pressure regulation have provided insight into the genetic basis of hypertension [reviewed in Sugiyama et al. (30)]. These studies have established the laboratory rat and mouse as powerful models for dissecting the genetic basis of essential hypertension.

Several of the rat and mouse hypertension QTL map to regions that contain homologous genes, suggesting that the genes determining hypertension are also homologous. Likewise, several of the limited number of human hypertension QTL also map to regions that contain genes homologous to those in rat and mouse hypertension QTL (28, 30). This concordance of hypertension QTL across species suggests that common allelic variations occur in only a subset of all the proteins that are involved in blood pressure regulation. These may be mutations in genes that code for rate-limiting or regulatory proteins since most proteins are present in excess, as shown by the large number of recessive diseases. The fact that QTL are concordant across species should help to guide the search for the genetic determinants of human hypertension, since many studies, such as narrowing a QTL region or carrying out expression studies, are more easily carried out in a rodent model.

In this study, we further exploited the mouse as a hypertension model. We conducted a QTL analysis of a population of (BALB/cJ × CBA/CaJ) F_2 males to identify genomic regions and gene-gene interactions that regulate blood pressure, heart rate, and heart weight. We identified several significant QTL: two associated with blood pressure, three significantly associated with heart rate, and one associated with heart weight.

Article published online before print. See web site for date of publication (http://physiolgenomics.physiology.org).

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METHODS

Mice. We obtained BALB/cJ (BALB) and CBA/CaJ (CBA) mice from the Jackson Laboratory (Bar Harbor, ME) and produced reciprocal F_1 populations (BALB × CBA; CBA × BALB) and 207 (BALB × CBA) F_2 males. We housed them in a room with a 14-h light/10-h dark cycle and gave them unrestricted access to acidified water and food (NIH31 6% fat open formula; Purina Mills, Richmond, IN). The cages were covered with a polyester filter and contained pine shavings bedding. The Institutional Animal Care and Use Committee at the Jackson Laboratory approved all animal protocols.

Phenotyping. To measure blood pressure and heart rate, we used a blood pressure analysis system (model BP-2000; Visitech Systems, Apex, NC). The machine accommodated four mice and consisted of a warming plate, which kept the mice at 38°C, and four restraining units (dark metal tunnels open at one end). Computer-automated tail cuffs placed on the tails of each mouse rapidly inflated and deflated, and blood pressure was detected by a photoresistor cell below each tail and recorded by a computer (14). The machine also recorded the heart rate of each mouse.

We reduced the variation in blood pressure by acclimating mice to the room for at least 2 wk, measuring their blood pressures when they were 8 wk (±1 wk) old, and taking all measurements in the morning (9–12 AM). Additionally, we trained the mice to the machine and all the associated measurement procedures for 5 days, designated as *days 1–5*, without recording blood pressures, then measured blood pressures on *days 8–12*, each day taking 20 measurements for a total of 100 measurements per mouse (expressed in mmHg as means ± SE). One of us (F. Sugiyama) carried out all blood pressure measurements. Mice were killed, and body and heart weights were determined. The numbers of mice are listed in Tables 1–3 and Figs. 1–5.

To ensure the quality of our data, we eliminated blood pressure readings that were less than 60 mmHg, because these low readings resulted from failure of the machine to detect the pulse of a mouse that was moving. If we were unable to successfully obtain at least eight pressure readings on a given day, then we did not use that day's data, since failure to obtain readings resulted from mice that were moving and probably were stressed. We used data from mice only if we were able to obtain at least 3 days of data. These criteria eliminated 12 of the 207 (6%) mice, providing a total of 195 mice for analysis. We eliminated blood pressure readings for a given mouse if these were more than 2 standard deviations from the mean for that mouse on that day. We used the same criteria for heart rate; if we were unable to obtain blood pressure, then we were unable to obtain heart rate.

Genotyping. We extracted DNA from the tail of each mouse (31) and genotyped it with 91 simple sequence length polymorphic (SSLP) markers spaced at ~15-cM intervals throughout the mouse genome; 11 additional SSLP markers were added in specific locations after we detected QTL (Fig. 1). We reported map positions in centimorgans using the 2001 Mouse Genome Informatics database (**http://www.informatics.jax.org**). Because most genetic information for a quantitative trait is at the extreme ends of its distribution in a population, we selected for genotyping the mice in the upper and lower 20% of each phenotypic trait. Because we measured so many traits, selecting the upper and lower 20% for each trait

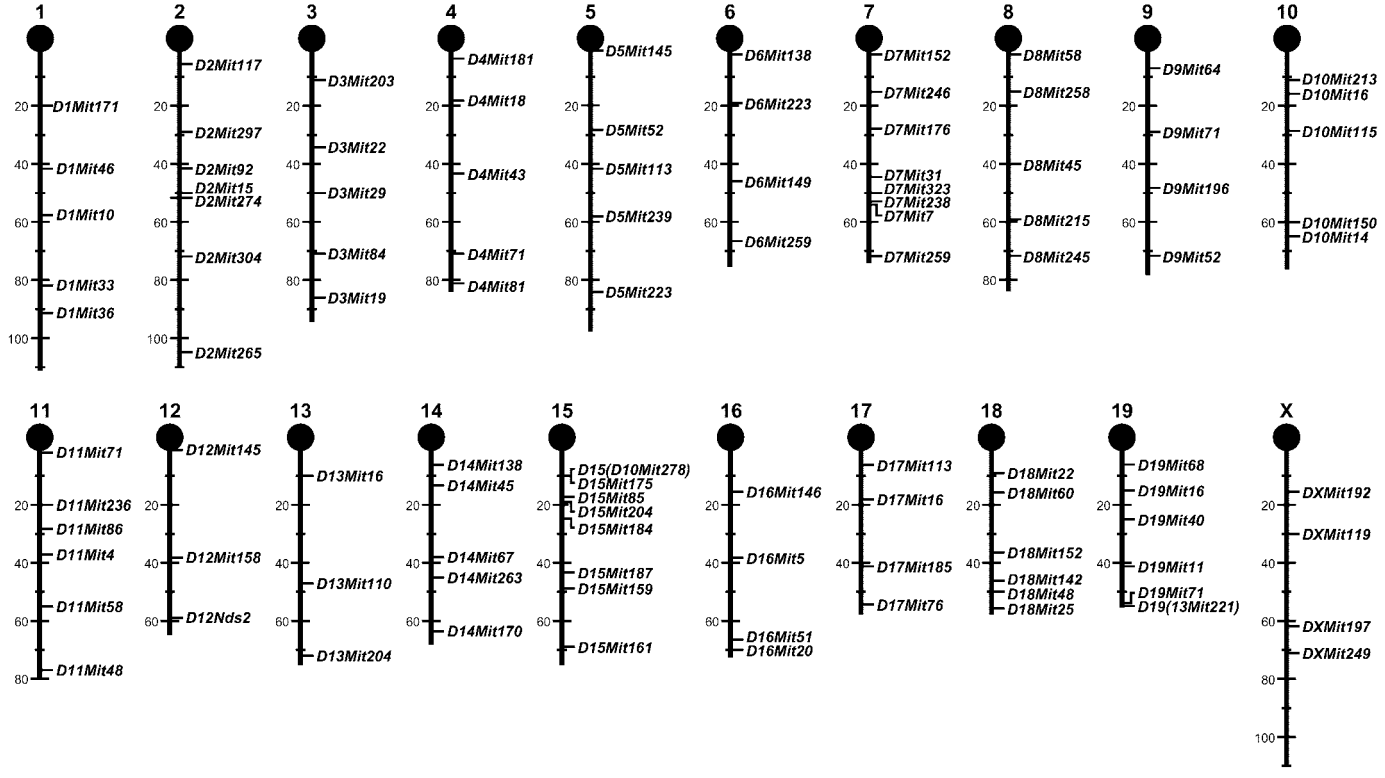

Fig. 1. Polymorphic markers used in the genome-wide scan. The markers are placed according to their positions in the Mouse Genome Database (2001) except for two markers that did not map to the correct place. These are *D10Mit278*, which mapped to the top of Chr 15, and *D13Mit221*, which mapped to the bottom of Chr 19; these are placed in the position they mapped in this cross. We do not know whether these markers were mapped incorrectly to begin with or whether we received primer pairs that were mislabeled from the supplier.

Table 1. *Systolic blood pressures, heart weights, and heart rates of CBA and BALB inbred mouse strains, and of F_1 and F_2 progeny*

Strains	Blood Pressure, mmHg	Heart Rate, beats/min	Heart Weight, mg
BALB	104 ± 2* (12)	484 ± 9 (12)	120 ± 2 (12)
CBA	96 ± 1 (11)	620 ± 7‡ (11)	108 ± 2‡ (12)
(BALB × CBA)F_1	106 ± 1† (11)	587 ± 10‡ (11)	138 ± 2‡ (12)
(CBA × BALB)F_1	110 ± 1† (5)	605 ± 16‡ (5)	155 ± 3‡ (8)
F_2	105 ± 1† (195)	610 ± 4‡ (195)	132 ± 4‡ (207)

Values are means ± SE; *n* is parentheses. *Significant difference ($P = 0.0001$) between CBA and other groups. †Significant difference ($P < 0.0001$) between CBA and other groups. ‡Significant difference ($P < 0.0001$) between BALB and other groups.

resulted in 162 of the 195 (83%) F_2 males being genotyped; all genotyped animals were used for the genome scan.

We purchased primers from Research Genetics (MapPairs, Huntsville, AL). The Allele Typing Service of the Jackson Laboratory genotyped the samples using fluorescent primers and the ABI model 3700 capillary electrophoresis apparatus. We reviewed the genotypes for the presence of double recombinants over short genetic distances, and questionable genotypings or missing data were repeated in our laboratory using standard procedures (17). The average spacing between markers was 15 cM. Four regions on chromosome (Chr) 10, 12, 13, and X had greater than 30 cM between markers. Since we found no polymorphic markers in these four regions (data not shown), we think these regions may be identical by descent because these two strains do share a common origin. Chromosome lengths were appropriate, and all markers fit a Hardy-Weinberg distribution.

Statistical analyses. To compare blood pressures between parental and F_1 mice, we used Student's *t*-test. To detect QTL significantly associated with blood pressure, heart rate, or heart weight, we analyzed genome-wide scans in three steps (29). In the first step, we identified main QTL associated with each phenotype by computing a logarithm of the odds ratio (LOD) score at 2-cM steps over the entire genome and compared these to significance thresholds computed by permutation analysis (4). Confidence intervals were computed by the method of Sen and Churchill (24) by finding the region under the posterior density curve (10^LOD) that contains 95% of the total area. In the second step, we identified pairs of loci significantly associated with a phenotype by conducting a simultaneous search for pairs analysis (24). Genome-wide scans were implemented in MATLAB software (Mathworks, Natick, MA; source codes available at **http://www.jax.org/research/churchill**). In the third step, we integrated all the main and interacting QTL-phenotype associations detected in the first two steps into a multiple regression (using SYS/STAT Software, version 8; SAS Institute, Cary, NC). F-statistics based on adjusted (type III) sums of squares were used to determine the contribution of a QTL in combination with all other QTL. These statistical methods have been described previously (24).

To assess the evidence for multiple linked QTL on the same chromosome, we calculated LOD scores for one, two, and three QTL models on each chromosome that showed a significant main effect. The difference in LOD score between the single and multiple QTL models was used to compute a likelihood ratio test (and thus a *P* value) for the evidence in favor of multiple linked QTL. These test statistics have nonstandard distributions (23), so we conducted simulations to estimate significance thresholds and *P* values. For the comparison of 2-QTL vs. 1-QTL models, the 95% significance threshold is 3.4 for additive QTL and 3.9 if we allow for interaction between the two QTL. The estimates are based on 1,000 simulated intercrosses with 200 mice and a single QTL with an effect size of 0.5 standard deviations. LOD scores for 3-QTL vs. 2-QTL models in this study were clearly nonsignificant, and no simulations were carried out.

RESULTS

Blood pressure, heart rate, and heart weight in parental strains, F_1 and F_2 progeny. The blood pressures of BALB mice were significantly higher than were

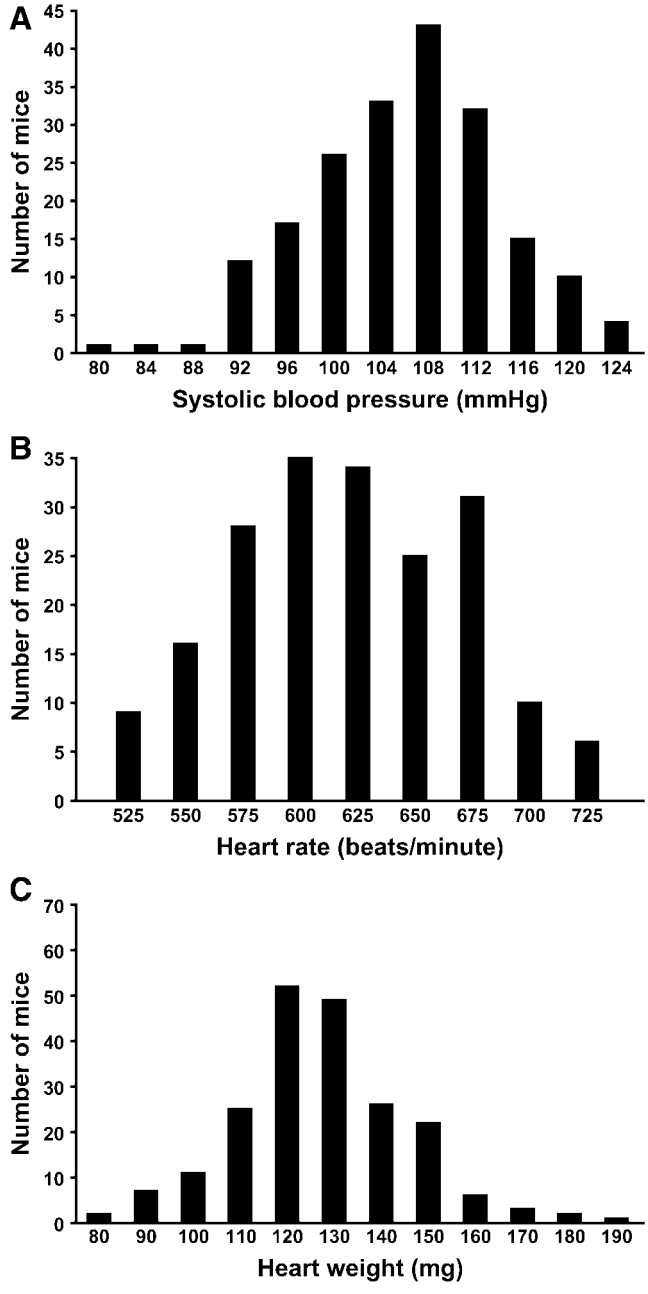

Fig. 2. Distribution of blood pressure (*A*), heart rate (*B*), and heart weight (*C*) in the F_2 male progeny. Blood pressure and heart rate are based on 100 measurements/mouse. Heart weight and body weight were obtained at the end of the experiment.

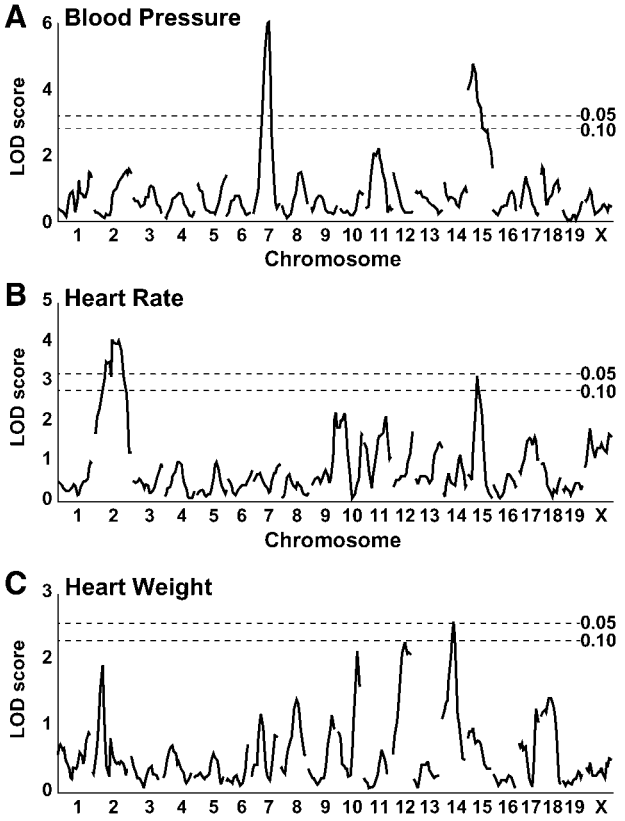

Fig. 3. Genome-wide scan of blood pressure (*A*), heart rate (*B*), and log of heart weight (*C*). Genome-wide suggestive ($P = 0.10$) and significant ($P = 0.05$) logarithm of the odds ratio (LOD) scores are shown as dotted lines and based on permutation testing.

those of CBA mice (104 ± 2 vs. 96 ± 1 mmHg, means ± SE; $P = 0.0001$, Table 1). This is not a large interstrain difference, but the wide distribution of blood pressures in the F_2 population suggested that genes were segregating and that QTL analysis would be successful. Blood pressure was similar in the reciprocal F_1 progeny ruling out maternal inheritance or imprinting. The genes underlying the lower blood pressure of CBA mice were apparently recessive because blood pressures of F_1 and F_2 progeny were more BALB-like than CBA-like.

The heart rates of BALB mice were significantly slower than those of CBA mice (484 ± 9 vs. 620 ± 7 beats/min, $P < 0.0001$, Table 1); those of the F_1 progeny were similar to each other and intermediate to the heart rates of the parental strains with a tendency to be more like CBA (Table 1). The hearts of BALB mice weighed significantly more than did those of CBA mice (120 ± 2 vs. 108 ± 2 mg, $P < 0.0001$, Table 1). The genes underlying the relatively slower heart rates and larger heart weights of BALB mice were probably recessive, because these phenotypes in F_1 and F_2 progeny were more CBA-like than BALB-like (Table 1). The mean blood pressures, heart rates, and heart weights of the F_2 progeny were distributed normally around the mean (Fig. 2).

Identification of QTL for blood pressure. The genome-wide scan indicated significant QTL for blood pressure on Chrs 7 and 15 (Fig. 3*A*). The Chr 15 QTL, already named *Bpq6* (for "blood pressure QTL 6") was previously found in two QTL crosses between strains A/J and C57BL/6J and between strains BPH and BPL [these latter strains are "blood pressure high" and "blood pressure low" strains developed by Schlager (22)]. However, in this cross the Chr 15 QTL is broad as shown by the interval map together with the 95%

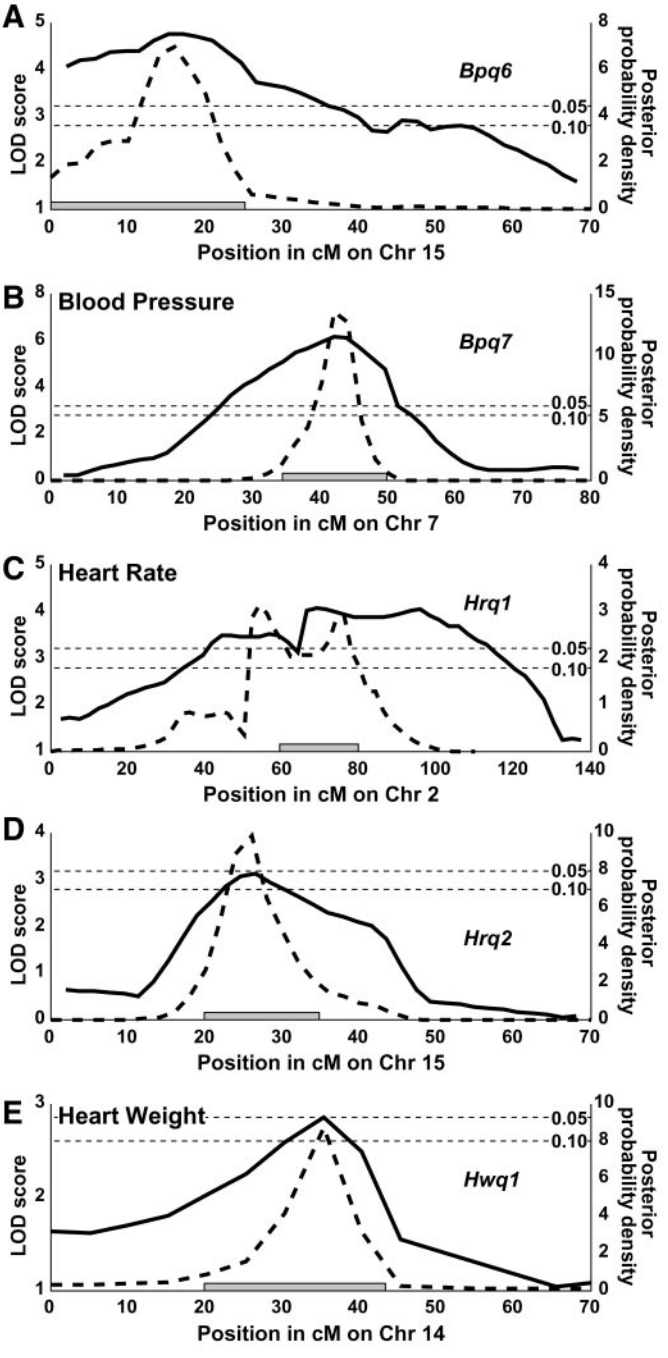

Fig. 4. Interval maps of quantitative trait loci (QTL) with 95% confidence limits for *Bpq6* (*A*), *Bpq7* (*B*), *Hrq1* (*C*), *Hrq2* (*D*), and *Hwq1* (*E*). The shape of the QTL is shown by the solid line, the 95% confidence limits as determined by posterior probability density are shown by the dashed line, and the suggestive ($P = 0.10$) and significant ($P = 0.05$) LOD scores are shown as dotted lines.

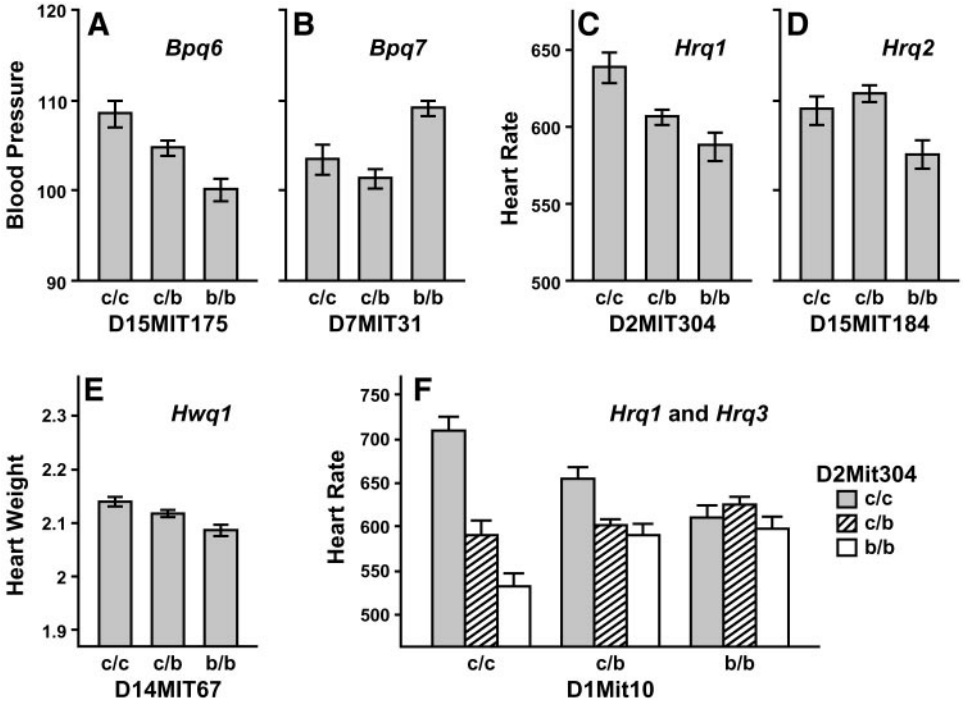

Fig. 5. Allele effects and interaction effects for QTL for *Bpq6* (*A*), *Bpq7* (*B*), *Hrq1* (*C*), *Hrq2* (*D*), and *Hwq1* (*E*). The allele effects are shown for homozygous CBA/CBA F_2 males (c/c), for heterozygous males (c/b), and for homozygous BALB/BALB males (b/b). *F*: interaction effects for Chr 1 and Chr 2 on heart rate is shown for F_2 males homozygous CBA/CBA at *D1Mit10* (c/c), heterozygous at *D1Mit10* (c/b), or homozygous BALB/BALB (b/b). The differing alleles at D2Mit304 are shown by gray (homozygous CBA/CBA), striped (heterozygous), or open (homozygous BALB/BALB) bars.

confidence interval (Fig. 4*A*). Confidence intervals were computed by the method of Sen and Churchill (24) by finding the region under the posterior density curve (10^LOD) that contains 95% of the total area. Equivalent results are obtained by the method of dropping down 1.5 units from the peak of the LOD curve. We calculated the LOD scores for the 1-QTL, 2-QTL, and 3-QTL models for Chr 15 without interaction effects. The difference in LOD scores for 2-QTL vs. 1-QTL models is 1.9 ($P = 0.51$) and is not statistically significant. We note, however, that this does not rule out the possibility of a second QTL on chromosome 15 (see DISCUSSION). The difference between the 2-QTL and the 3-QTL model is a LOD score of 0.5, which is not significant.

The Chr 7 QTL was also found previously in the A/J × C57BL/6 cross as an interaction (24); it is now named *Bpq7*. The interval map for *Bpq7* and the 95% confidence interval, as determined by posterior probability density (24), is shown in Fig. 4*B*. The allele effects of *Bpq6* and *Bpq7* are shown in Fig. 5, *A* and *B*. The *Bpq6* allele for high blood pressure came from strain CBA, and the *Bpq7* allele for high blood pressure came from strain BALB.

The scan for interacting QTL did not reveal any for blood pressure in this cross. The multiple linear regression (Table 2) shows that the Chr 7 and 15 QTL accounted for 22.5% of the variance. Table 3 lists the chromosomal location, LOD scores, allele giving the highest value, and peak markers for each blood pressure QTL and for the heart rate and heart weight QTL discussed below.

Identification of QTL for heart rate and heart weight. The genome-wide scan revealed significant QTL for heart rate on Chrs 2 and 15 (Fig. 3*B*). The interval maps for these QTL and the 95% confidence intervals are shown in Fig. 4, *C* and *D*. The allele effects of the Chr 2 QTL, named *Hrq1* (for "heart rate QTL"), and the Chr 15 QTL, named *Hrq2*, are shown in Fig. 5, *C* and *D*. In both cases strain CBA contributed the allele for high heart rate; the CBA allele is additive for *Hrq1* but dominant for *Hrq2*. This is consistent with the heart rates in F_1 progeny (Table 1), which show that heart rate is between the two parents but tends to be more like CBA. *Hrq2* on Chr 15 appears to be different than *Bpq6* on Chr 15; their 95% confidence intervals overlap only slightly (0–25 cM for *Bpq6*; 20–35 cM for *Hrq2*).

The Chr 2 QTL is broad with a distinct cusp (Fig. 4*C*). We calculated the LOD scores for the single QTL

Table 2. *Multiple regression analysis*

Source	DF	Type III SS	*F* Value	*P*
Blood pressure				
D7Mit31	2	1,530	13.38	<0.0001
D15Mit175	2	1,036	9.06	0.0002
Total	152	11,392		
Variance explained		22.5%		
Heart rate				
D2Mit304	2	7.99	16.84	<0.0001
D15Mit184	2	2.45	5.16	0.0069
D1Mit10	2	0.04	0.09	0.91
D2Mit304∗*D1Mit10*	4	5.09	5.37	0.0005
Total	154	48.41		
Variance explained		32.2%		
Heart weight				
D12Mit158	2	0.0128	1.76	0.18
D12Nds2	2	0.0226	3.10	0.048
D14Mit67	2	0.0522	7.15	0.0011
Total	159	0.6712		
Variance explained		13.1%		

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

Table 3. *Chromosomal location, peak marker, confidence interval, and LOD score for blood pressure, heart rate, and heart weight QTL*

Trait	Name	Chr	Peak, cM	95% CI, cM	High Allele	Peak Marker	LOD
Blood pressure	*Bpq7*	7	42	35–50	BALB	*D7Mit31*	6.1
Blood pressure	*Bpq6*	15	16	0–25	CBA	*D15Mit175*	4.9
Heart rate	*Hrq1*	2	72	60–80	CBA	*D2Mit304*	4.0
Heart rate	*Hrq2*	15	26	20–35	CBA	*D15Mit184*	3.1
Heart rate	*Hrq3*	1	60	45–75	CBA	*D1Mit10*	0.2*
Heart weight	*Hwq1*	14	38	20–43	BALB	*D14Mit67*	2.9

LOD, logarithm of the odds ratio score; QTL, quantitative trait locus; Chr, chromosome; CI, confidence interval. **Hrq3* has no significant effect by itself but the interaction between *Hrq1* and *Hrq3* is significant. The confidence intervals for *Bpq6* and *Hrq1* are not reliable, since both of these are on chromosomes with complex loci.

and the two QTL model for Chr 2; the difference between the 1-QTL LOD and the 2-QTL LOD with interaction is 3.15 ($P = 0.19$). Although the test failed to achieve the standard 0.05 level of significance, we interpret this as suggestive evidence for multiple QTL. The maximal peak for a single QTL on Chr 2 occurs at 72 cM and we name this QTL *Hrq1*. If a second locus exists, it would localize somewhere in the region 40–80 cM, so it is tightly linked. The two loci appear to interact epistatically, but with tight linkage not all genotype combinations occur in the cross, and it is difficult to characterize the interaction.

The search for gene-gene interaction showed a significant interaction between *D1Mit10* (57 cM) and *D2Mit304* for heart rate. We named this interacting QTL on Chr 1 *Hrq3*. The magnitude of the interaction between *Hrq3* and *Hrq1* (Fig. 5*F*) shows that heart rate for mice with a *D1Mit10* CBA allele in either a homozygous or heterozygous state are very affected by the alleles at *D2Mit304* with the CBA allele contributing to higher heart rate. For mice that are homozygous for the BALB allele at *D1Mit10*, the *Hrq3* locus has no effect. The multiple regression analysis for heart rate (Table 2) shows that these three QTL account for 32.2% of the variance in heart rate.

The genome scan for heart weight was carried out on the log of heart weight and also on the log of heart weight adjusted with body weight as a covariate. The two genome scans gave equivalent results; the scan for log heart weight is shown (Fig. 3*C*). One significant QTL was found at *D14Mit67* with the peak at cM 38 and the 95% confidence interval at 20–43 cM (Fig. 4*E*); we name this QTL *Hwq1* for "heart weight QTL 1." Two additional QTL that reach suggestive significance were also found, both on Chr 12. These were not named because they are only suggestive, but both were included in the multiple regression analysis since we used a high threshold for suggestive. The allele effects of *Hwq1* are shown in Fig. 5*E*, and the regression analysis is shown in Table 2. The significant locus on Chr 14, and the two suggestive loci on Chr 12 account for 13.1% of the variance.

DISCUSSION

Several studies have recently conducted QTL analyses of progeny from crosses between inbred mouse strains that differed in blood pressure and have identified genomic regions associated with polygenic hypertension. Wright et al. (33) conducted QTL analyses of strains inbred for blood pressure differences (BPH for high blood pressure and BPL for low blood pressure). Analysis of the (BPH/2 × BPL/1)F_2 progeny and the progeny of two crosses of (BPL/1 × *Mus spretus*) identified 10 QTL associated with systolic blood pressure, and one associated with ventricular mass. We (29) conducted a QTL analysis of (C57BL/6J × A/J) F_1 × C57BL/6J progeny and found six QTL associated with salt-sensitive hypertension. Three of the QTL we identified for salt-induced hypertension appeared to be the same as those identified by Wright and colleagues (Ref. 33): *Bpq1* (25–60 cM on Chr 1), *Bpq5* (66–80 cM on Chr 6), and *Bpq6* (0–30 cM on Chr 15).

In our current study, we conducted a QTL analysis of (CBA × BALB) F_2 progeny. Ordinarily, the blood pressure difference of 8 mmHg between the two parental strains with standard deviations in the range of 5 would not be sufficient to justify a QTL analysis, since this represents a strain difference of less than 2 standard deviation units. However, we planned this cross for other purposes, and the wide distribution of blood pressure among F_2 progeny encouraged us to search for QTL for this trait. We identified two QTL associated with blood pressure, *Bpq6* and *Bpq7*. The fact that BALB and CBA each contributed one allele for high blood pressure may explain the small interstrain blood pressure difference between the two parents compared with the difference in the F_2 population. We identified the effect of *Bpq6* on blood pressure in a previous study (29) as an interacting QTL; *Bpq6* had an effect on blood pressure through its interaction with *Bpq5* on Chr 6. We have assumed that *Bpq6* found in this cross and the previous studies identify the same gene, although further data may reveal that these are distinct genes. *Bpq7* was also found in our previous cross as an interacting QTL between A/J and C57BL/6, although we did not report it in our original paper (29). It was found and reported later as our methods for finding interacting QTL improved (24). We also identified three QTL significantly associated with heart rate; *Hrq1*, *Hrq2*, and *Hrq3* and one associated with heart weight, *Hwq1*.

Two of the QTL (*Bpq6* and *Hrq1*) reported in this study appear to consist of multiple loci. We feel that it is important to report indications that a QTL region may harbor multiple loci, because it is our experience

that upon further dissection (e.g., by analysis of congenics) such QTL may break apart into multiple independent effects. The lack of strong statistical support for multiple QTL is likely to be a consequence of low power to detect linked QTL. Although we can provide some indication of the complexity of these QTL using statistical indices, it is difficult to precisely localize multiple effects in an intercross population of this size.

Bpq6, *Bpq7*, *Hrq1*, and *Hrq3* each harbor promising candidate genes. We previously reported that *Bpq6* contains two candidate genes; prostaglandin receptor gene *Pterep2* and *Npr3* (natriuretic peptide receptor 3; cM 6.7). However, further mapping studies showed that *Pterep2* was really on Chr 14 and the prostaglandin receptor on Chr 15 was a different member of the same gene family, *Ptgerep4* (prostaglandin E receptor EP4 subtype; cM 6.4). Like the *Ptgerep2*, *Ptgerep4* codes for a G protein-coupled receptor, and a number of studies support its role in blood pressure regulation (1–3, 7, 20). The most promising candidate genes for *Bpq7* are *Adm* (adrenomedullin; cM 50.5), which encodes a potent vasodilator peptide that exerts major effects on cardiovascular function (21); *Pth* (parathyroid hormone; cM 52.5), *Calc* (calcitonin, α-calcitonin gene-related peptide; cM 54), which shares some structural homology with *Adm* and is a potent vasodilator (2, 20), and *Scnn1b* and *1g* (sodium channel, non-voltage-gated 1β and 1γ; cM 56). Mice that lack calcitonin develop hypertension (8). *Scnn1b* and *Scnn1g*, the sodium channel, non-voltage-gated 1β or 1γ, participate in the control of sodium flux in the kidney; mutations in these proteins have been associated with Liddle syndrome, a rare form of monogenic human hypertension (9, 26). Some interesting candidate genes for heart rate are located on Chrs 1 and 2. Acetylcholine is known to affect heart rate (3), and two classes of cholinergic receptors are known; the muscarinic receptors, of which five are known, and the nicotinic receptors, which exists as a pentamer composed of four different subunits. The muscarinic receptors are G-coupled proteins and are known to affect heart rate; the nicotinic receptor is a ligand-gated ion channel that affects sodium and potassium flux and may affect heart rate (3, 10, 11, 25, 32). *Chrna1* (cholinergic receptor, nicotinic, polypeptide-α) is located on Chr 2 at cM 43, and two other subunits of this receptor are located on Chr 1 at cM 52.3 (*Chrnd*, *Chrng*; cholinergic receptor, nicotinic, polypeptides δ and γ). These subunits and one other form a pentamer that is the receptor. Two of the muscarinic receptors are also located on Chr 2 in the *Hrq1* region, *Chrm4* and *Chrm5* (cholinergic receptor, muscarinic, 4 and 5; cM 49 and 58).

Evidence for the concordance of QTL across species is increasing. Previously, several studies demonstrated that a QTL in one experimental animal model can predict the location of a QTL in a homologous region in another species, especially humans (13, 28, 29). A recent review provides evidence for additional QTL that are concordant in mice, rats, and humans (12). Our current study offers more evidence for this type of concordance: both murine hypertension QTL we identified were concordant with blood pressure QTL in humans. *Bpq6* is homologous to human 5p12–14 and 8q22–24; a human hypertension QTL has been found in both of these regions region (7, 19). *Bpq7* is homologous to three human chromosomal regions: cM 35–44 is homologous to human 15q23–26; cM 44–54 are homologous to human 11p12–13 and 11q13–21; and cM 55–56 are homologous to human 16p12–13. A human hypertension QTL has been found for 15q25.1–26.1 (16, 34), and a rare form of monogenic human hypertension, Liddle syndrome, maps to 16p12–13 (9, 26). The rat homology to *Bpq6* is not known except for a 2-cM region homologous to rat 2; this small region of rat Chr 2 is part of a hypertension QTL (32). Homology to rat Chr 7 begins at the distal edge of *Bpq6* at cM 25; this region of rat Chr 7 does contain a hypertension QTL (5, 6), but the most likely candidate gene *Cyp11b1* (7) maps to mouse cM 44, which is clearly out of the *Bpq6* region. Since the Chr 15 QTL appears to be complex, there may be a second QTL on Chr 15 which is homologous to rat 7, but it is not the region we define as *Bpq6*. *Bpq7* is homologous to rat Chr 1, which does contain a QTL for hypertension (32). A QTL for heart rate has been found in rats (15); its location on rat Chr 3 has a peak homologous to mouse Chr 2, cM 35. This is not the location of *Hrq1*, which is at cM 72, but we note that the Chr 2 locus appears to be complex and may have a second peak about cM 40; this second peak is homologous with the rat QTL.

These results establish the laboratory mouse as an excellent model for essential hypertension. Further work in additional inbred mouse strains will more clearly characterize quantitative blood pressure and other cardiovascular traits and enable us to elucidate the genes that regulate these traits in humans.

We thank Olga Savinova, Harry Whitmore, and Janice Martin for excellent technical assistance and Ray Lambert for technical writing skills.

This work was supported by grants from the National Heart, Lung, and Blood Institute's Program for Genomic Applications (HL-66611) and the SCOR program (HL-55001), by the American Heart Association Grant 50564N (G. A. Churchill and R. Li), by the American Health Assistance Foundation G1999023, and by the Japanese Ministry of Education, Science, Sport, Culture, and Technology (F. Sugiyama). S. W. M. John is an assistant investigator of the Howard Hughes Medical Institute.

REFERENCES

1. **Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Targher G, Alberiche M, Bonadonna RC, and Muggeo M.** Prevalence of insulin resistance in metabolic disorders: the Bruneck Study. *Diabetes* 47: 1643–1649, 1998.
2. **Breimer LH, MacIntyre I, and Zaidi M.** Peptides from the calcitonin genes: molecular genetics, structure and function. *Biochem J* 255: 377–390, 1988.
3. **Brown JH and Taylor P.** Muscarinic receptor agonists and antagonists. In: *The Pharmacological Basis of Therapeutics* (9th ed.), edited by Hardman JG and LE Limbird. New York: McGraw-Hill, 1996, p. 141–160.
4. **Churchill GA and Doerge RW.** Empirical threshold values for quantitative trait mapping. *Genetics* 138: 963–971, 1994.
5. **Cicila GT, Garrett MR, Lee SJ, Liu J, Dene H, and Rapp JP.** High-resolution mapping of the blood pressure QTL on chromosome 7 using Dahl rat congenic strains. *Genomics* 72: 51–60, 2001.

6. **Cicila GT, Rapp JP, Wang JM, St Lezin E, Ng SC, and Kurtz TW.** Linkage of 11 beta-hydroxylase mutations with altered steroid biosynthesis and blood pressure in the Dahl rat. *Nat Genet* 3: 346–353, 1993.
7. **Curnow KM, Slutsker L, Vitek J, Cole T, Speiser PW, New MI, White PC, and Pascoe L.** Mutations in the CYP11B1 gene causing congenital adrenal hyperplasia and hypertension cluster in exons 6, 7, and 8. *Proc Natl Acad Sci USA* 90: 4552–4556, 1993.
8. **Gangula PR, Zhao H, Supowit SC, Wimalawansa SJ, Dipette DJ, Westlund KN, Gagel RF, and Yallampalli C.** Increased blood pressure in alpha-calcitonin gene-related peptide/calcitonin gene knockout mice. *Hypertension* 35: 470–475, 2000.
9. **Hansson JH, Nelson-Williams C, Suzuki H, Schild L, Shimkets R, Lu Y, Canessa C, Iwasaki T, Rossier B, and Lifton RP.** Hypertension caused by a truncated epithelial sodium channel gamma subunit: genetic heterogeneity of Liddle syndrome. *Nat Genet* 11: 76–82, 1995.
10. **Hassall CJ, Stanford SC, Burnstock G, and Buckley NJ.** Co-expression of four muscarinic receptor genes by the intrinsic neurons of the rat and guinea-pig heart. *Neuroscience* 56: 1041–1048, 1993.
11. **Hoover DB, Baisden RH, and Xi-Moy SX.** Localization of muscarinic receptor mRNAs in rat heart and intrinsic cardiac ganglia by in situ hybridization. *Circ Res* 75: 813–820, 1994.
12. **Jacob HJ and Kwitek AE.** Rat genetics: attaching physiology and pharmacology to the genome. *Nat Rev Genet* 3: 33–42, 2002.
13. **Julier C, Delepine M, Keavney B, Terwilliger J, Davis S, Weeks DE, Bui T, Jeunemaitre X, Velho G, Froguel P, Ratcliffe P, Corvol P, Soubrier F, and Lathrop GM.** Genetic susceptibility for human familial essential hypertension in a region of homology with blood pressure linkage on rat chromosome 10. *Hum Mol Genet* 6: 2077–2085, 1997.
14. **Krege JH, Hodgin JB, Hagaman JR, and Smithies O.** A noninvasive computerized tail-cuff system for measuring blood pressure in mice. *Hypertension* 25: 1111–1115, 1995.
15. **Kreutz R, Struk B, Stock P, Hübner N, Ganten D, and Lindpaintner K.** Evidence for primary genetic determination of heart rate regulation. Chromosomal mapping of a genetic locus in the rat. *Circulation* 96: 1078–1081, 1997.
16. **Krushkal J, Ferrell R, Mockrin SC, Turner ST, Sing CF, and Boerwinkle E.** Genome-wide linkage analyses of systolic blood pressure using highly discordant siblings. *Circulation* 99: 1407–1410, 1999.
17. **Mu JL, Naggert JK, Svenson KL, Collin GB, Kim JH, McFarland C, Nishina PM, Levine DM, Williams KJ, and Paigen B.** Quantitative trait loci analysis for the differences in susceptibility to atherosclerosis and diabetes between inbred mouse strains C57BL/6J and C57BLKS/J. *J Lipid Res* 40: 1328–1335, 1999.
18. **Rapp JP.** Genetic analysis of inherited hypertension in the rat. *Physiol Rev* 80: 135–172, 2000.
19. **Rice T, Rankinen T, Province MA, Chagnon YC, Perusse L, Borecki IB, Bouchard C, and Rao DC.** Genome-wide linkage analysis of systolic and diastolic blood pressure: the Quebec Family Study. *Circulation* 102: 1956–1963, 2000.
20. **Rosenfeld MG, Mermod JJ, Amara SG, Swanson LW, Sawchenko PE, Rivier J, Vale WW, and Evans RM.** Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. *Nature* 304: 129–135, 1983.
21. **Sakata J, Shimokubo T, Kitamura K, Nakamura S, Kangawa K, Matsuo H, and Eto T.** Molecular cloning and biological activities of rat adrenomedullin, a hypotensive peptide. *Biochem Biophys Res Commun* 195: 921–927, 1993.
22. **Schlager G.** Biometrical genetic analysis of blood pressure level in the genetically hypertensive mouse. *Clin Exp Hypertens* 16: 809–824, 1994.
23. **Self SG and Liang KY.** Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under non-standard conditions. *J Am Statist Assoc* 82: 605–610, 1987.
24. **Sen S and Churchill GA.** A statistical framework for quantitative trait mapping. *Genetics* 159: 371–387, 2001.
25. **Shi H, Wang H, and Wang Z.** Identification and characterization of multiple subtypes of muscarinic acetylcholine receptors and their physiological functions in canine hearts. *Mol Pharmacol* 55: 497–507, 1999.
26. **Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, Gill JR Jr, Ulick S, Milora RV, Findling JW, Canessa CM, Rossier BC, and Lifton RP.** Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. *Cell* 79: 407–414, 1994.
27. **Smith IK.** The pressure's on. A hypertension drug taken by 28 million people is under scrutiny What are the other options? *Time* 156: 126, 2000.
28. **Stoll M, Kwitek-Black AE, Cowley AW Jr, Harris EL, Harrap SB, Krieger JE, Printz MP, Provoost AP, Sassard J, and Jacob HJ.** New target regions for human hypertension via comparative genomics. *Genome Res* 10: 473–482, 2000.
29. **Sugiyama F, Churchill GA, Higgins DC, Johns C, Makaritsis KP, Gavras H, and Paigen B.** Concordance of murine quantitative trait loci for salt-induced hypertension with rat and human loci. *Genomics* 71: 70–77, 2001.
30. **Sugiyama F, Yagami Ki K, and Paigen B.** Mouse models of blood pressure regulation and hypertension. *Curr Hypertens Rep* 3: 41–48, 2001.
31. **Taylor BA and Rowe L.** Genes for serum amyloid A proteins map to chromosome 7 in the mouse. *Mol Gen Genet* 195: 491–499, 1984.
32. **van Koppen CJ, Lenz W, and Nathanson NM.** Isolation, sequence and functional expression of the mouse m4 muscarinic acetylcholine receptor gene. *Biochim Biophys Acta* 1173: 342–344, 1993.
33. **Wright FA, O'Connor DT, Roberts E, Kutey G, Berry CC, Yoneda LU, Timberlake D, and Schlager G.** Genome scan for blood pressure loci in mice. *Hypertension* 34: 625–630, 1999.
34. **Xu X, Rogus JJ, Terwedow HA, Yang J, Wang Z, Chen C, Niu T, Wang B, Xu H, Weiss S, Schork NJ, and Fang Z.** An extreme-sib-pair genome scan for genes regulating blood pressure. *Am J Hum Genet* 64: 1694–1701, 1999.