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Abstract—The genetic basis of hypertension is well established, yet very few genes that cause common forms of hypertension are known. Quantitative trait locus (QTL) analyses in rodent models can guide the search for human hypertension genes, but the excellent genetic resources for mice have been underused in this regard. To address this issue, we surveyed blood pressure variation in mice from 37 inbred strains and generated 2577 mice in 8 intercross populations to perform QTL analyses of blood pressure. We identified 14 blood pressure QTL in these populations, including ≥7 regions of the mouse genome not linked previously to blood pressure. Many QTL were detected in multiple crosses, either within our study or in studies published previously, which facilitates the use of bioinformatics methods to narrow the QTL and focus the search for candidate genes. The regions of the human genome that correspond to all but 1 of the 14 blood pressure QTL in mice are linked to blood pressure in humans, suggesting that these regions contain causal genes with a conserved role in blood pressure control. These results greatly expand our knowledge of the genomic regions underlying blood pressure regulation in mice and support future studies to identify the causal genes within these QTL intervals. **(*Hypertension*. 2009;54:802-809.)**

Key Words: mouse ■ tail cuff ■ blood pressure ■ quantitative trait locus ■ concordance

Blood pressure is a highly heritable phenotype affected by multiple genes and environmental factors. The genetic basis of hypertension has been investigated extensively in humans through genome-wide and candidate-gene association studies, as well as genome-wide linkage analyses. Despite the substantial effort made to identify genes underlying polygenic hypertension (see Cowley[1] for review), few causal genes have been identified to date.

One alternative approach to studying the genetic basis of hypertension in humans is to identify genes affecting blood pressure in model organisms, mainly rodents, and then test those genes for a role in human blood pressure control. A common strategy for identifying genomic regions linked to a phenotype in rodent models is quantitative trait locus (QTL) analysis, and rodent blood pressure QTL often correspond with regions of the human genome containing genes affecting blood pressure.[2,3] This finding suggests that the same genes may be linked to blood pressure control in humans and rodents. In fact, parallel studies in humans and rats successfully identified the genes encoding adducin[4] and 11β-hydroxylase[5] as important in blood pressure control. Recently, Chang et al[6] combined human linkage analysis with published mouse linkage and haplotype analysis[7] to identify 9 candidate genes on human chromosome (Chr) 1q that they tested for association with blood pressure in humans; 2 of the 3 genes significantly associated with blood pressure are within the mouse haplotype region. These findings support the approach of using animal models to identify genes affecting blood pressure and then translating the findings to humans through association studies.

The resources available for genetic mapping in mice are exemplary, yet rats are the preferred rodent model for blood pressure QTL analysis. The rat genome database (www.rgd.mcw.edu) lists 292 blood pressure–related QTL in rats, but only 13 QTL linked to blood pressure in mice. Therefore, we performed QTL analyses of blood pressure in 8 mouse intercross populations to better understand the genetic regulation of blood pressure in mice and to facilitate comparative genomic mapping between mice and humans.

Methods

Breeding and Phenotyping Inbred Mice for the Strain Survey of Blood Pressure

Mice from 37 inbred strains were purchased from The Jackson Laboratory (Bar Harbor, ME), Clea Japan (Tokyo, Japan), or Charles River Japan (Yokohama, Japan) and bred at the Laboratory Animal Resource Center, University of Tsukuba. Tail-cuff systolic blood pressures (SBPs) were measured using a BP-98A blood pressure system (Softron; please see the online Data Supplement at http://

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From the Cardiovascular and Metabolism Disease Area, Novartis Institutes for BioMedical Research and Novartis Pharmaceuticals Corp (M.F., J.T., L.X., K.D.), East Hanover, NJ; The Jackson Laboratory (M.E.D., R.K., S.-W.T., R.D., G.A.C.), Bar Harbor, Maine; Laboratory Animal Resource Center (T.I., F.S.), University of Tsukuba, Tsukuba, Japan.

Correspondence to Keith DiPetrillo, Novartis Institute for BioMedical Research, 1 Health Plaza, Building 437, Room 4331, East Hanover, NJ 07936. E-mail keith.dipetrillo@novartis.com

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Table 1. Characteristics of 8 F_2 Populations for Blood Pressure QTL Analysis

Grandmaternal Strain	Grandpaternal Strain	n	No. of Markers	SBP Difference*	*P*†
129S1/SvImJ (129)	A/J (A)	336	91	7.0	0.010
129S1/SvImJ (129)	DBA/2 (DBA)	324	90	5.9	0.019
AKR/J (AKR)	NZW/LacJ (NZW)	334	94	14.3	<0.001
BTBR T+tf (BTBR)	SWR/J (SWR)	336	93	25.7	<0.001
C3H/HeJ (C3H)	KK/HlJ (KK)	335	91	11.3	<0.001
FVB/NJ (FVB)	RIIIS/J (RIII)	252	90	5.3	0.020
PL/J (PL)	CBA/J (CBA)	324	90	13.4	<0.001
SJL/J (SJL)	RIIIS/J (RIII)	336	91	14.7	0.002

Strain abbreviations used throughout the article are shown in parentheses.

*Data show the differences in SBPs between the strains (millimeters of mercury).

†Data show the *P* values for blood pressure differences between inbred strains.

hyper.ahajournals.org for details). All of the blood pressure measurements were taken from 10-week–old male mice in the morning, and the values from 100 successful readings (20 readings on each of 5 consecutive days) per mouse were used to calculate individual averages. Study protocols were approved by the university animal experimental committee of the University of Tsukuba.

Breeding and Phenotyping F_2 Populations

Mice from 14 inbred strains were purchased from The Jackson Laboratory and bred at Novartis Pharmaceuticals Corp to generate 8 F_2 populations for QTL analysis (summarized in Table 1). All of the F_1 mice for each cross were generated in the same direction and intercrossed to produce the F_2 progeny, meaning that maternal, imprinting, and mitochondrial effects were fixed within each F_2 population. Tail-cuff blood pressure was measured in 8-week–old male F_2 mice using a CODA-6 noninvasive blood pressure monitoring system (Kent Scientific). The accuracy of the CODA-6 system has been validated by comparison with simultaneous telemetry measurements,[8] and we determined that a training week was not required for this system (Figure S1, available in the online Data Supplement). All of the measurements were taken in the afternoon, and values from ≤100 measurement cycles (20 per day for 5 days) were used to calculate average SBPs and SDs for each mouse. Any reading >2 SD from the mean for an individual mouse was discarded, and final averages and SDs were recalculated. Only mice having a final average SBP calculated from ≥40 cycles, of 100 cycles maximum, were used for the QTL analyses.

Genotyping

DNA was isolated by phenol:chloroform extraction from the tail of each F_2 mouse and genotyped by KBiosciences with ≈90 single-nucleotide polymorphism markers evenly spaced across the genome.[9] This number of single-nucleotide polymorphism markers provides similar power to detect and resolve QTL as an infinite number of markers.[10]

QTL Analyses

To minimize the influence of extreme phenotype values on the QTL analyses, SBP values were converted to van der Waerden normal scores within each cross.[11] A 3-step analysis[12] was used to identify QTL linked to blood pressure. QTL mapping was performed in R/qtl.[13] Because the single-nucleotide polymorphism markers used for genotyping are mapped to physical positions in the genome, centimorgan (cM) positions were approximated by dividing megabase positions by 2 for genetic mapping; we confirmed the validity of this approximation by comparison with the cM positions estimated from the genotype data from each cross. Main-effect QTLs were identified by calculating logarithm of the odds scores at 2-cM intervals across the genome and compared with genome-wide adjusted significance ($P<0.05$) thresholds calculated by permutation testing.[12,14] CIs were determined as 95% of the area under the posterior probability density curves. QTL from each cross were fit to a multiple regression model to assess their effects on blood pressure. Ultimately, all of the QTL were mapped to the physical mouse genome map.

Statistics

The Tukey honestly significant differences test was used to test the significance of unplanned, pairwise comparisons among the 37 inbred strains. Values for groups sorted by genotype are presented as mean±SE and were compared by ANOVA followed by Bonferroni posttest using SigmaStat. $P<0.05$ was considered significant.

Results

Survey of SBP in Inbred Mice

To evaluate SBP among inbred mice and identify strains useful for QTL analysis, we measured SBP in mice from 37 different inbred strains. The strain survey data, with individual values, is publicly available in the Mouse Phenome Database (http://www.jax.org/phenome). We

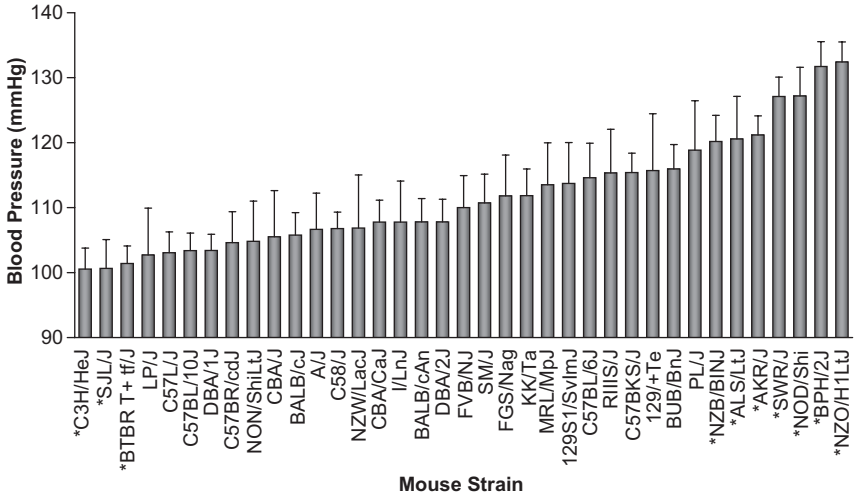

Figure 1. SBPs in mice from 37 inbred strains. Strains marked with the same letter were intercrossed to produce F_2 populations for linkage analysis. Bars represent tail-cuff blood pressures for mice from each strain given as mean±SD. Strains marked with an asterisk are significantly different ($P<0.05$ by Tukey honestly significantly differences test) from the median strain (ie, FVB). Please see Table S1 for a summary of all of the significant differences between the strains.

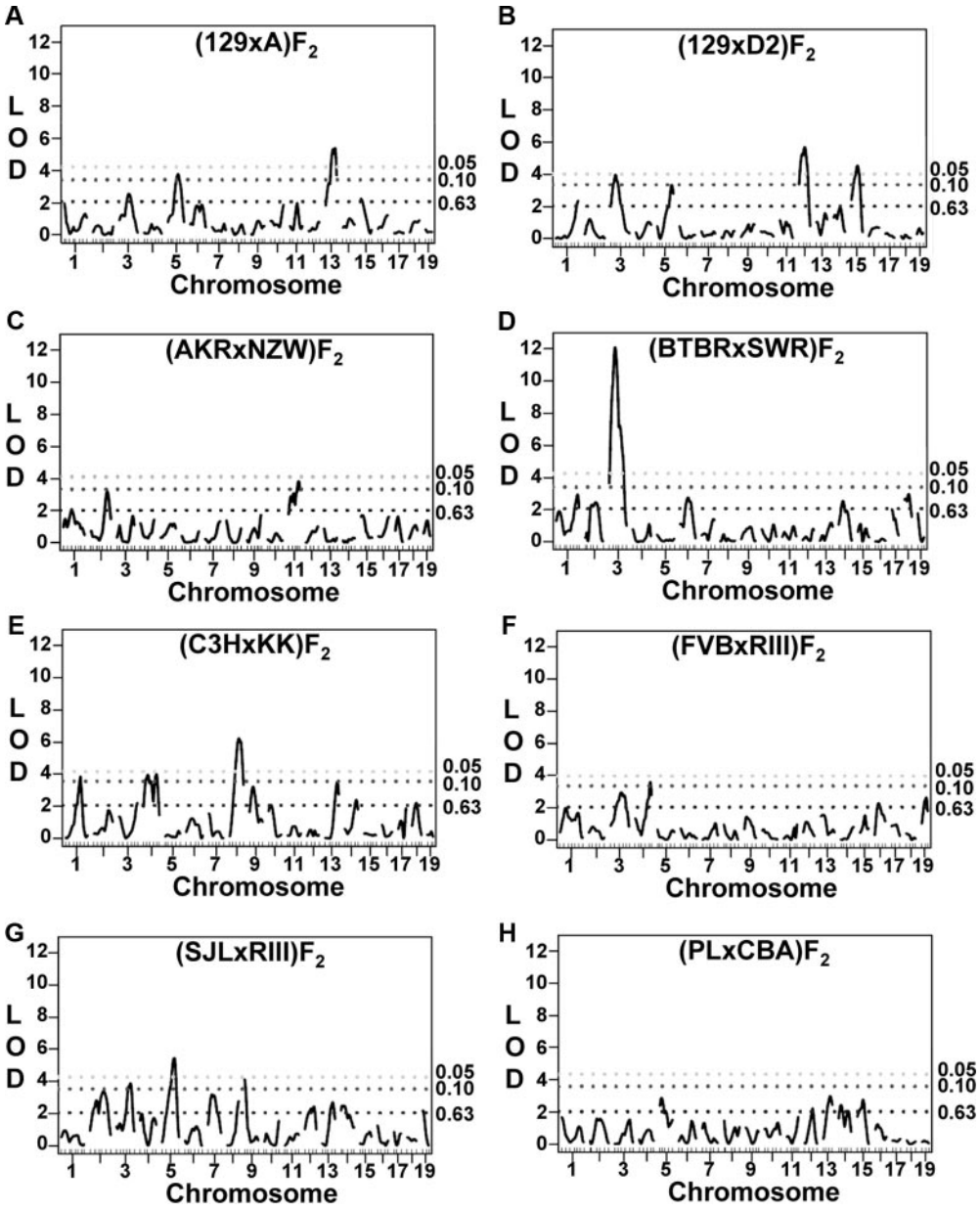

Figure 2. Genome-wide scans for blood pressure QTL in 8 intercross populations. Suggestive ($P<0.10$) and significant ($P<0.05$) logarithm of the odds ratios (LOD) scores, as determined by permutation testing, are shown as dotted lines.

found a wide variation in SBP between mice from different inbred strains, from C3H mice with SBP at ≈100 mm Hg to NZO mice with SBP >130 mm Hg (Figure 1; please see Table S1).

QTL Analyses of SBP in F_2 Populations

Based on the strain survey data and genetic diversity between inbred mouse strains, we chose mice from 12 inbred strains to generate 8 F_2 populations for QTL analyses. Although none of the strains are considered hypertensive (SBP >140 mm Hg), SBP was significantly different between each of the strain pairs used to generate the F_2 populations (Table 1), and each F_2 population displayed a wide blood pressure distribution (Figure S2). We performed QTL analysis on these 8 F_2 populations and detected significant, main-effect QTL in all but 1 of the populations (Figure 2); the (PL×CBA)F_2 population did not identify any QTL significantly linked to SBP. From the 7 analyses, 14 regions of the mouse genome were linked to SBP on 10 different chromosomes (peak locations, CIs, allele effects, logarithm of the odds scores, and modes of inheritance are summarized in Table 2).

Chr 1

We detected a significant QTL on Chr 1 affecting SBP in the (C3H×KK)F_2 population (Figure 3A). Mice that inherited either 2 C3H or KK alleles at this locus had significantly higher blood pressure than heterozygous mice, indicating an overdominant pattern of inheritance (Figure 3B).

Chr 3

Chr 3 was significantly linked to SBP in 3 of the F_2 populations tested, and the mapping plots suggest the pres-

Table 2. Summary of Significant, Main-Effect QTL Linked to SBP

Chr	QTL Name	Cross	Peak, cM	95% CI, cM	95% CI, Mb	LOD	High Allele	BP Effect, mm Hg*	Mode of Inheritance	Human QTL
1	*Bpq24*	C3H×KK	66.2	52.6 to 80.6	106.4 to 161.8	3.85	?	3.3	Over-Dom	1q31, 2q34
3	*Bpq16*	129×D2	21.8	3.8 to 40.6	7.5 to 80.5	3.96	D2	9.4	Rec	3q24–26, 4q31
	Bpq20	BTBR×SWR	27.8	19.6 to 43.6	39.4 to 86.5	12.10	SWR	17.1	Add	3q24–26, 4q31
	Bpq28	SJL×RIIIS	61.8	43.6 to 76.1	86.5 to 151.6	3.88	RIII	7.9	Rec	1p13
4	*Bpq25*	C3H×KK	57.6	3.6 to 69.1	7.7 to 139.8	3.98	C3H	9.0	Dom	1p33–34, 6q14
	Bpq27	FVB×RIIIS	65.2	44.5 to 69.1	90.9 to 139.8	3.59	RIII	7.6	Rec	1p33–34
5	*Bpq14*	129×A	42.5	22.5 to 67.4	46.4 to 136.5	3.77	A	10.0	Dom	4p
	Bpq29	SJL×RIIIS	54.2	40.1 to 73.5	88.7 to 148.3	5.46	RIII	11.3	Dom	4p
8	*Bpq26*	C3H×KK	42.6	31.0 to 62.5	65.7 to 128.0	6.22	KK	12.9	Add	4q32, 16q12, 19p13
9	*Bpq30*	SJL×RIIIS	6.7	6.7 to 18.9	13.2 to 37.4	4.13	RIII	11.2	Add	11q24, 19p13
11	*Bpq19*	AKR×NZW	46.1	6.1 to 51.5	12.2 to 102.0	3.82	AKR	7.8	Rec	2p14, 5q34, 17p13
12	*Bpq17*	129×D2	30.1	8.1 to 52.1	16.2 to 110.4	5.69	129	10.7	Add	–
13	*Bpq15*	129×A	44.9	23.9 to 51.0	48.2 to 105.6	5.39	129	12.7	Rec	5q33, 5q13
15	*Bpq18*	129×D2	28.6	4.6 to 48.7	9.0 to 96.4	4.53	129	10.6	Dom	5p14, 8q24

Mb indicates megabase; LOD, logarithm of the odds ratio; BP, blood pressure; Rec, recessive; Add, additive; Dom, dominant.
*BP effect is the maximum blood pressure difference between the 3 genotypes at the QTL.

ence of 2 blood pressure QTL on this chromosome (Figure 3C). Proximal Chr 3 was linked to SBP in the (129×D2)F_2 and (BTBR×SWR)F_2 populations. Although D2 mice contributed a recessive high blood pressure allele on Chr 3 (Figure 3D), SWR mice contributed an additive high blood pressure allele at this locus (Figure 3E). The CIs for these QTL were proximal to 45 cM, but Chr 3 distal to 45 cM was linked to SBP in the (SJL×RIII)F_2 population (Figure 3C). Mice that inherited 2 RIII alleles on distal Chr 3 showed significantly higher blood pressure values than those that inherited 1 or 2 SJL alleles (Figure 3F). The mapping plot of Chr 3 for the (BTBR×SWR)F_2 cross indicated that Chr 3 distal to 45 cM may also be linked to SBP in this population (Figure 3C).

Chr 4

The (C3H×KK)F_2 population identified a significant QTL affecting SBP that spanned Chr 4 (Figure 4A). C3H mice contributed a recessive high blood pressure allele at this QTL (Figure 4B). Distal Chr 4 (≈45 to 60 cM) was also linked to

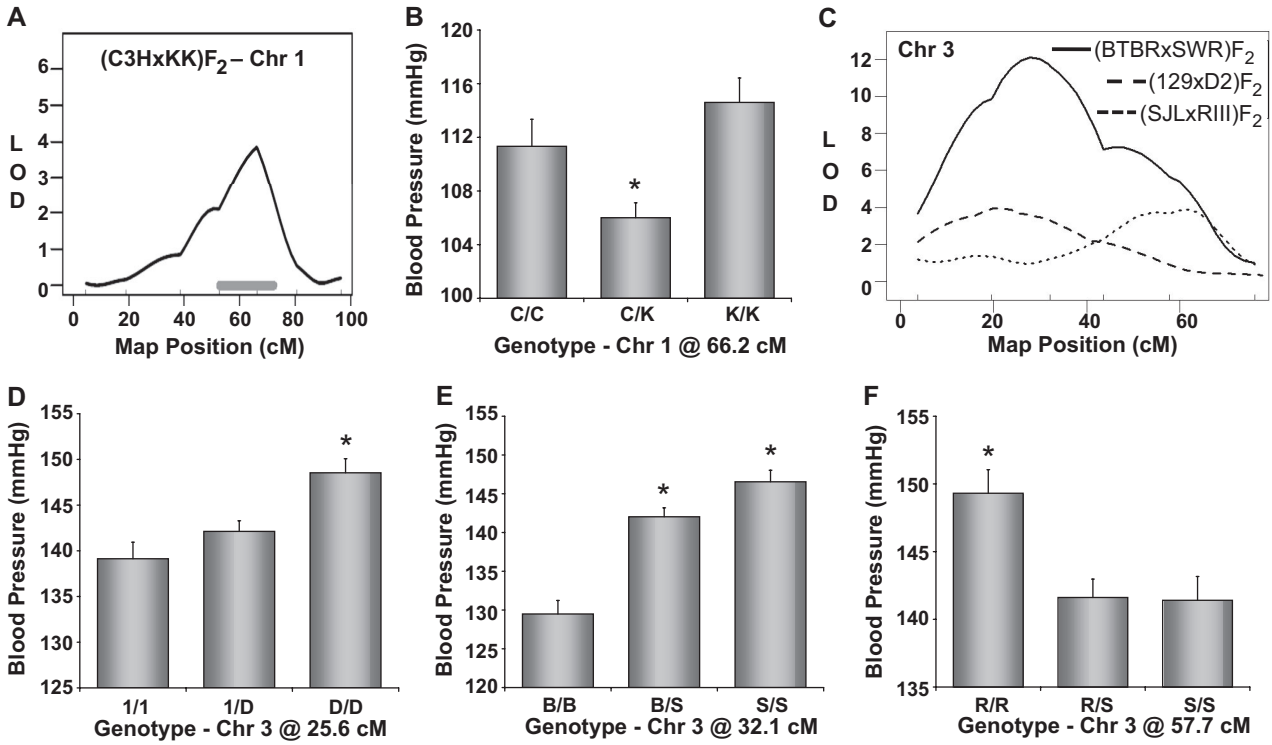

Figure 3. Mapping of blood pressure QTL on Chrs 1 and 3. Mapping plot (A) and allelic effect plot (B) for the Chr 1 QTL in the (C3H×KK)F_2 population. The solid, horizontal gray bar represents the Bayesian CI. C, Mapping plots for the Chr 3 QTL detected in the (BTBR×SWR)F_2, (129×D2)F_2, and (SJL×RIII)F_2 populations. The corresponding allelic effect plots of tail-cuff blood pressure are shown in D through F. Blood pressure values are expressed as mean±SEM. *$P<0.05$ vs all of the other genotypes.

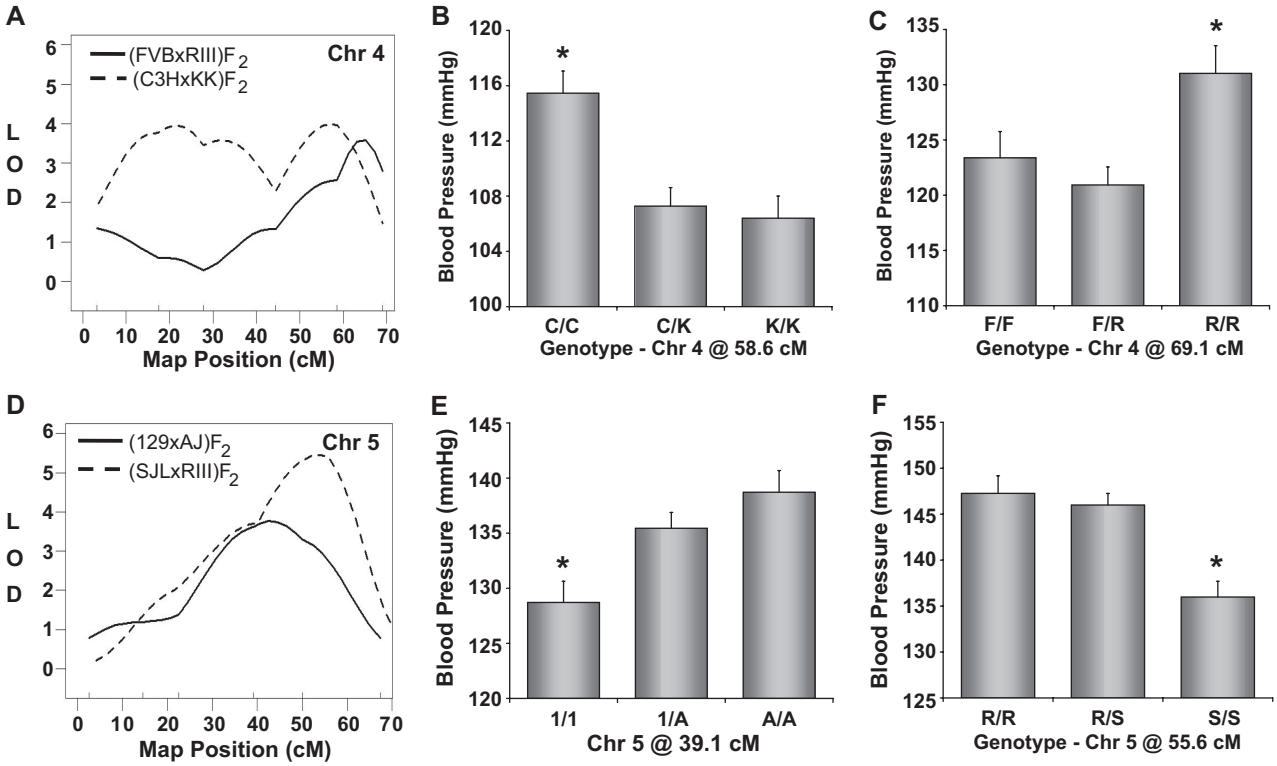

Figure 4. Mapping of blood pressure QTL on Chrs 4 and 5. Mapping plots for individual QTL are shown on the left and the corresponding allelic effect plots of tail-cuff blood pressure are shown on the right. Blood pressure values are expressed as mean±SEM. *$P<0.05$ vs all of the other genotypes.

SBP in the (FVB×RIII)F_2 population, where a recessive high blood pressure allele was inherited from RIII mice (Figure 4C). The (C3H×KK)F_2 mapping plot and distal Chr 4 QTL in the (FVB×RIII)F_2 population suggest the presence of multiple blood pressure QTLs on Chr 4.

Chr 5

Chr 5 was significantly linked to SBP in both the (129×A)F_2 and (SJL×RIII)F_2 crosses (Figure 4D). SJL and 129 contributed recessive high blood pressure alleles in their respective populations (Figure 4E and 4F).

Chr 8

Distal Chr 8 contained a significant QTL underlying SBP in the (C3H×KK)F_2 intercross (Figure 5A). At this locus, KK mice contributed an additive high blood pressure allele (Figure 5B).

Chr 9

A narrow interval of proximal Chr 9 was significantly linked to SBP in the (SJL×RIII)F_2 population (Figure 5C). SJL mice contributed an additive low blood pressure allele at this locus (Figure 5D).

Chr 11

The Chr 11 mapping plot for the (AKR×NZW)F_2 population shows a broad SBP QTL spanning from 6 to ≈52 cM (Figure 5E). At this locus, AKR mice contributed a recessive high blood pressure allele (Figure 5F).

Chr 12

The middle of Chr 12 was significantly linked to SBP in the (129×D2)F_2 population (Figure 6A), where 129 mice contributed an additive high blood pressure allele (Figure 6B).

Chr 13

Distal Chr 13 contained a significant SBP QTL in the (129×A)F_2 intercross (Figure 6C). F_2 mice that inherited two 129 alleles at this locus displayed significantly higher SBP than heterozygotes or A homozygotes (Figure 6D), indicating a recessive 129 high blood pressure allele at this QTL.

Chr 15

The final SBP QTL detected in our analyses of 8 mouse intercross populations was on Chr 15 (Figure 6E). In the (129×D2)F_2 population, this QTL was inherited as a recessive high blood pressure allele from D2 mice (Figure 6F).

Discussion

Considering the limitations of available methods for blood pressure measurement in mice, we chose to use tail-cuff manometry because QTL analyses require high-throughput blood pressure phenotyping. We previously validated the accuracy of the CODA-6 tail-cuff system for measuring SBP by comparison with simultaneous radiotelemetry blood pressure measurements.[8] Blood pressure can be greatly affected by environmental conditions and measurement technique; despite substantial environmental and methodologic differences, results from the strain survey performed at the University of Tsukuba enabled us to produce 7 effective mapping populations at Novartis. Within our QTL analyses, we carefully controlled time of measurement (afternoon only), ambient conditions (ie, temperature and noise), and operator handling to limit measurement variability. To further reduce variability, we

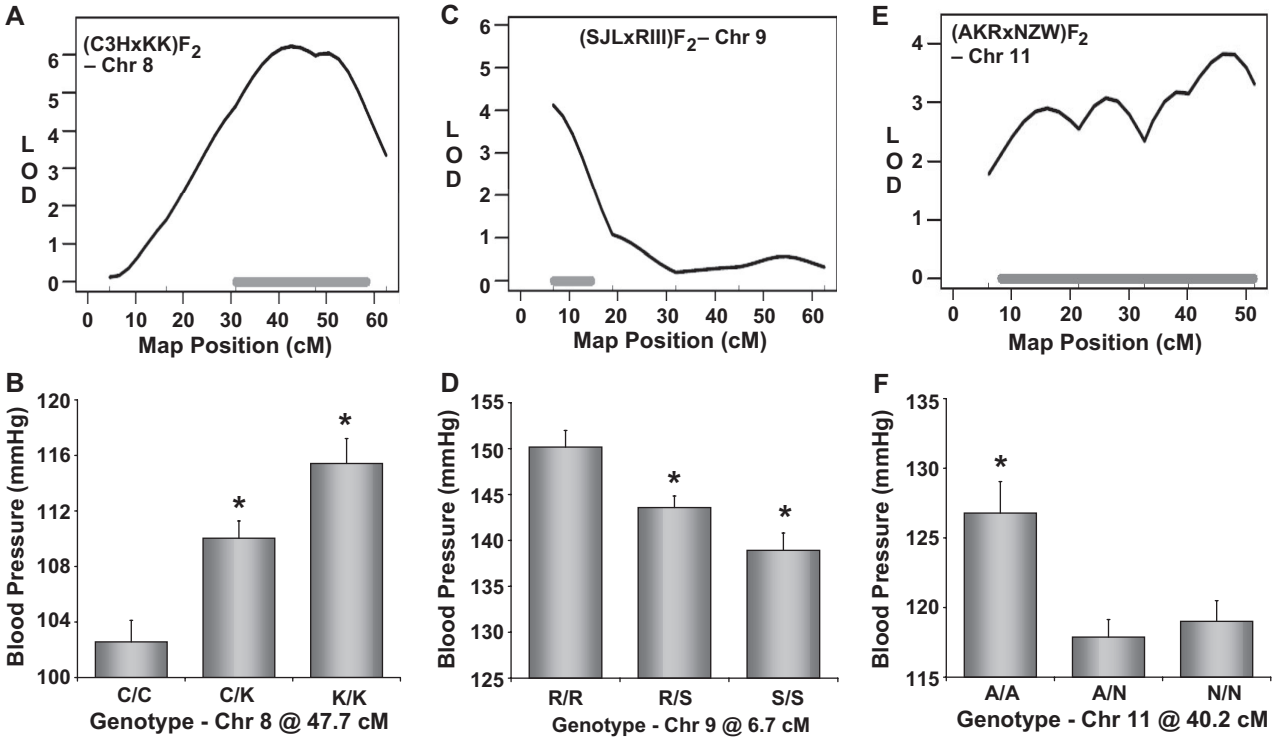

Figure 5. Mapping of blood pressure QTL on Chrs 8, 9, and 11. Mapping plots for individual QTL are shown on the left, and the corresponding allelic effect plots of tail-cuff blood pressure are shown on the right. The solid, horizontal gray bars in the mapping plots represent Bayesian CIs. Blood pressure values are expressed as mean±SEM. *$P<0.05$ vs all of the other genotypes.

conducted 5 daily measurement sessions of 20 measurements each and calculated the average blood pressure from ≤100 discrete measurements selected to exclude outliers >2 SD from the initial mean. We believe that this experimental strategy provides an accurate measurement of blood pressure in each mouse, and this approach has been used previously for QTL analyses of blood pressure in mice.[2,7,15]

In this study, we conducted QTL analyses of 8 intercrosses generated with mice from 14 inbred strains. Although none of the 14 inbred mouse strains used is considered hypertensive, each strain pair used to produce an F_2 population had a significant difference in SBP (Table 1), and even strain pairs without significantly different SBPs can be used to identify significant QTL affecting blood pressure. For example, Sugiyama et al[15] identified significant, main-effect blood pressure QTL in a (BALB×CBA)F_2 intercross. BALB and CBA each contributed 1 high blood pressure allele at the main-effect loci,[15] which could account for the significant blood pressure QTL in the F_2 population despite no difference in blood pressure between these inbred mice. In our crosses, we detected significant QTL in F_2 populations generated from inbred mice with SBP differences as low as 5.3 mm Hg. Overall, the number of QTL detected was not proportional to the phenotypic difference between the parental strains. Strain pairs with large SBP differences (BTBR versus SWR: 25.7 mm Hg; PL versus CBA: 13.4 mm Hg) produced F_2 populations that identified only 1 significant QTL between them, whereas the (129×D2)F_2 population identified 3 significant QTL despite the small SBP difference (5.9 mm Hg) between the parental strains.

Some of the QTL identified in our intercrosses replicate blood pressure QTL detected previously in other mouse crosses (Figure 7). For example, we identified a significant QTL on Chr 15 in the (129×D2)F_2 intercross that overlaps a QTL found previously in crosses between CBA and BALB mice,[15] A and B6 mice,[2] and BPH and BPL mice.[16] The concordant regions of the rat and human genomes have also been linked to blood pressure.[17–19] Other previously reported blood pressure QTL on distal Chr 1,[2,7,20] proximal Chr 4,[2,20,21] and Chr 8[20] were replicated in our studies. In addition to these previously identified QTL, we also identified many novel blood pressure QTL in the 8 intercross populations (Figure 7). Proximal Chr 3 was linked to SBP in (129×D2)F_2 and (BTBR×SWR)F_2 populations, whereas distal Chr 3 was linked in the (SJL×RIIIS)F_2 population. Distal Chrs 4 and 5 also contained novel QTL detected in multiple crosses, whereas Chrs 12 and 13 were each linked to SBP in a single intercross population.

Moreno et al[22] found that kidney weight:body weight ratio can serve as an intermediate phenotype for blood pressure QTL on the basis of correlation between the phenotypes and QTL concordance. However, only 1 blood pressure QTL corresponded with a kidney weight QTL in the same cross (Table S2), suggesting that kidney weight is not an intermediate phenotype for blood pressure QTL in mice.

Blood pressure QTL identified in both mice and rats are often concordant with human blood pressure QTL.[2,3] The

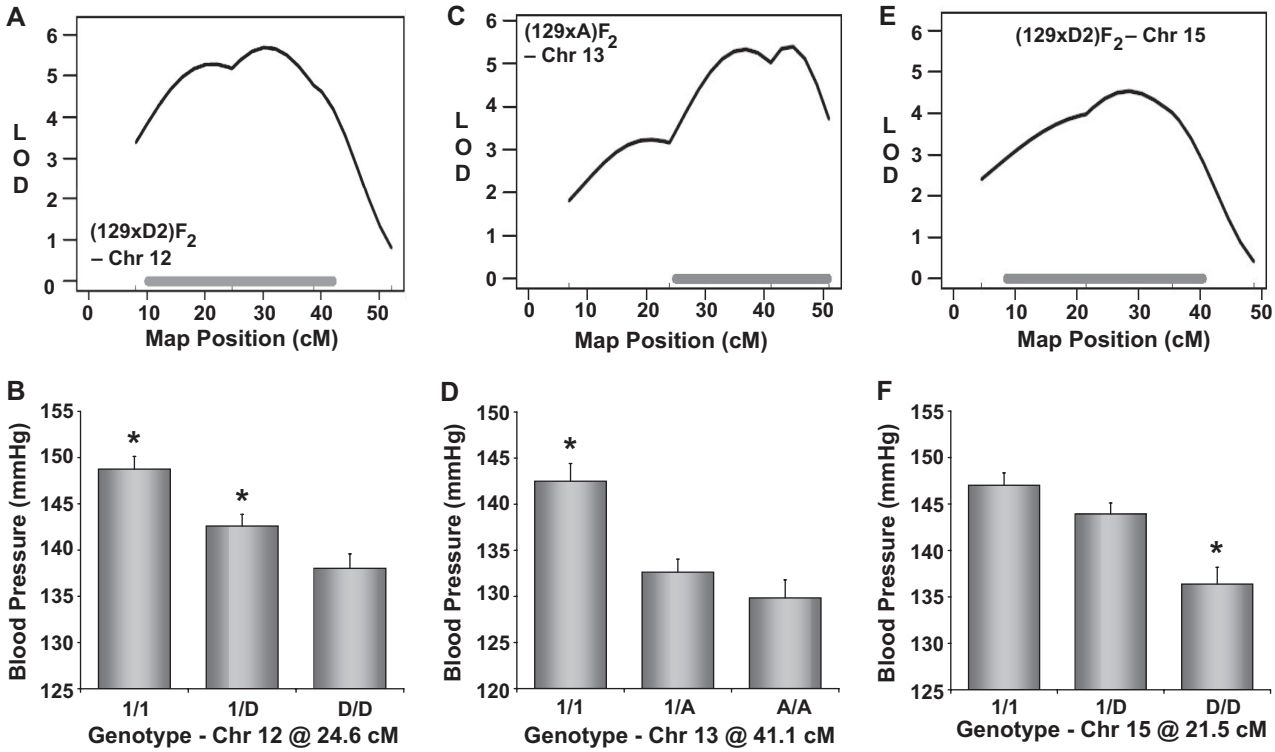

Figure 6. Mapping of blood pressure QTL on Chrs 12, 13, and 15. Mapping plots for individual QTL are shown on the left, and the corresponding allelic effect plots of tail-cuff blood pressure are shown on the right. The solid, horizontal gray bars in the mapping plots represent Bayesian CIs. Blood pressure values are expressed as mean±SEM. *$P<0.05$ vs all of the other genotypes.

regions of the human genome corresponding to all but 1 of the QTL identified in the intercross populations that we analyzed contained blood pressure QTL (Table 2; see Cowley[1] for review of human hypertension QTL). This high degree of concordance between mouse and human blood pressure QTL suggests evolutionary conservation of genes affecting blood pressure.

Perspectives

The 8 mouse populations examined in our study double the number of populations used for linkage analysis of blood pressure. The majority of QTL detected in our study have been replicated, either within these 8 intercross populations or in published studies; however, several QTL were detected in only 1 cross, suggesting that future crosses may detect additional new blood pressure QTL. New QTL crosses investigating the genetic basis of blood pressure may also replicate these QTL and provide more information for interval-specific haplotype analysis.[23] Future studies to fine map these QTL and identify the causal genes could elucidate novel pathways affecting blood pressure in both mice and humans.

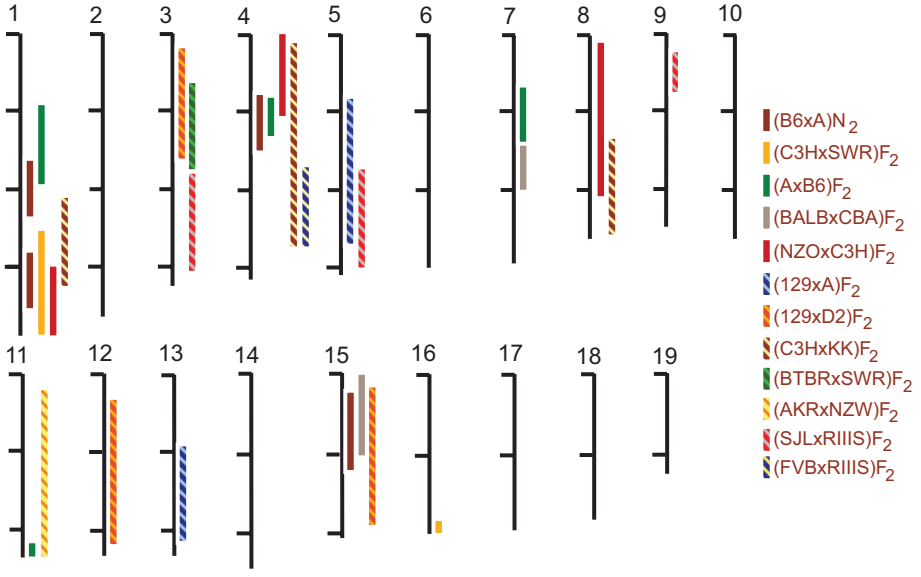

Figure 7. Summary of mouse blood pressure QTL. Solid bars represent blood pressure QTL intervals from published studies. Hatched bars indicate blood pressure QTL identified in the present study. Chromosomes are segmented in 50-megabase intervals indicated by horizontal tick marks.

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Disclosures

None.

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Online Supplement:

Genetic analysis of blood pressure in eight mouse intercross populations

Minjie Feng[1], Marion E. Deerhake[2], Ryan Keating[2], Jill Thaisz[1], Lingfei Xu[1], Shirng-Wern Tsaih[2], Randy Smith[2], Taichiro Ishige[3], Fumihiro Sugiyama[3], Gary A. Churchill[2], and Keith DiPetrillo[1].

[1]Novartis Institutes for BioMedical Research & Novartis Pharmaceuticals Corp., East Hanover, NJ 07936
[2]The Jackson Laboratory, Bar Harbor, ME 04609
[3]Laboratory Animal Resource Center, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan

Corresponding Author:
Keith DiPetrillo, Ph.D.
Novartis Institute for BioMedical Research
1 Health Plaza, Bldg. 437, Room 4331
East Hanover, NJ 07936
Phone: 862-778-0275
Fax: 973-781-2507
Email: keith.dipetrillo@novartis.com

EXPANDED MATERIALS AND METHODS:

Breeding and Phenotyping Inbred Mice for the Strain Survey of Blood Pressure: Mice from 37 inbred strains were purchased from either The Jackson Laboratory (Bar Harbor, ME), Clea Japan (Tokyo, Japan), or Charles River Japan (Yokohama, Japan) and bred at the Laboratory Animal Resource Center, University of Tsukuba. Mice were housed in plastic cages (2–5 per cage), under a 14-h light:10-h dark cycle, and had free access to a commercial chow diet (NMF; Oriental Company, Ltd., Tokyo, Japan) and autoclaved water. All study protocols were approved by the University Animal Experimental Committee of the University of Tsukuba.

Tail-cuff systolic blood pressures (SBP) were measured using a BP-98A blood pressure system (Softron, Tokyo, Japan). Each mouse was wrapped, with its tail protruding, in a cotton sheet and inner cover and warmed in a restrainer at 37°C. Tail pulse waves were monitored with a sensor attached to a tail-cuff and the mice were allowed to acclimate to the restrainer until pulse waves were gentle and rhythmic. After the acclimation period, blood pressures were measured and recorded automatically by computer. All blood pressure measurements were taken in the morning, and the values from 100 successful readings (20 readings on each of five consecutive days) per mouse were used to calculate individual averages. The strain survey data, with individual values, is publicly available in the Mouse Phenome Database (http://www.jax.org/phenome).

Breeding and Phenotyping F_2 Populations: Mice from fourteen inbred strains were purchased from The Jackson Laboratory and bred at Novartis Pharmaceuticals Corp. to generate eight F_2 populations for QTL analysis (summarized in Table 1). All of the F_1 mice for each cross were generated in the same direction and intercrossed to produce the F_2 progeny, meaning that maternal, imprinting, and mitochondrial effects were fixed within each F_2 population. We chose to use large F_2 populations so that we could detect recessive QTL donated from either parental strain and so that we could discriminate recessive, additive, and dominant effects. All mice were housed in cages with Enrich-O'-Cobs bedding (The Andersons Inc., Maumee, OH), fed with Harlan Tecklad Rodent Diet (#8604; Madison, WI), given free access to water with a reverse osmosis automatic watering system, and maintained on a 12 hour light/dark cycle.

Blood pressure was measured in 8-week-old, F_2 mice by tail-cuff manometry using a CODA-6 non-invasive blood pressure monitoring system (Kent Scientific, Torrington, CT). The accuracy of the CODA-6 system has been validated by comparison to simultaneous telemetry measurements (1). The mice were restrained in a plastic tube restrainer, occlusion and volume-pressure recording (VPR) cuffs were placed over their tails, and the mice were allowed to adapt to the restrainer for 5 minutes prior to initiating the blood pressure measurement protocol. After the 5 minute adaptation period, blood pressure was measured for 10 acclimation cycles followed by 20 measurement cycles. Mice were warmed by heating pads during the acclimation cycles to ensure sufficient blood flow to the tail. The animals were monitored closely throughout the measurement protocol, individually heated or cooled as necessary, and removed from restraint as soon as possible upon completing the measurement protocol. All measurements were taken in the afternoon. This animal protocol was reviewed and approved by the Novartis Animal Care and Use Committee.

Previous QTL analyses of blood pressure using mice employed a training week followed by a measurement week to collect the blood pressure data for analysis (2-4). The primary purpose of training was to acclimate the mice to the system and measurement procedure before collecting data, but the 10 acclimation cycles performed each day, during both the training and measurement weeks, could also serve this purpose. Because we employed a training week for our first two QTL analyses in the (129xD2)F_2 and (129xA)F_2 populations, we compared the results from the training and measurement weeks from a subset of 218 (129xA)F_2 and 244 (129xD2)F_2 mice to determine whether the training week was effective. Bland-Altman analysis (5) showed an average difference of -0.1 mmHg between final SBP from the training and measurement weeks (Figure S1). Moreover, the average difference in SBP standard deviation between the training and measurement weeks was 0.1 mmHg (Figure S1). Although the final average SBP and the variability in SBP for an individual mouse were not different between the weeks, there were an average of three more successful readings per mouse during the measurement week compared to the training week. Because the training week did not substantially improve the results during the measurement week, the training week was not used for the remaining 6 crosses.

The values from up to 100 measurement cycles (20/day x 5 days) were used to calculate average systolic blood pressures (SBP) and standard deviations (SD) for each mouse. Any reading greater than two SD from the mean for an individual mouse was discarded and final averages and SD were re-calculated. Only mice having a final average systolic blood pressure calculated from at least 40 cycles, out of 100 cycles maximum, were used for the QTL analyses.

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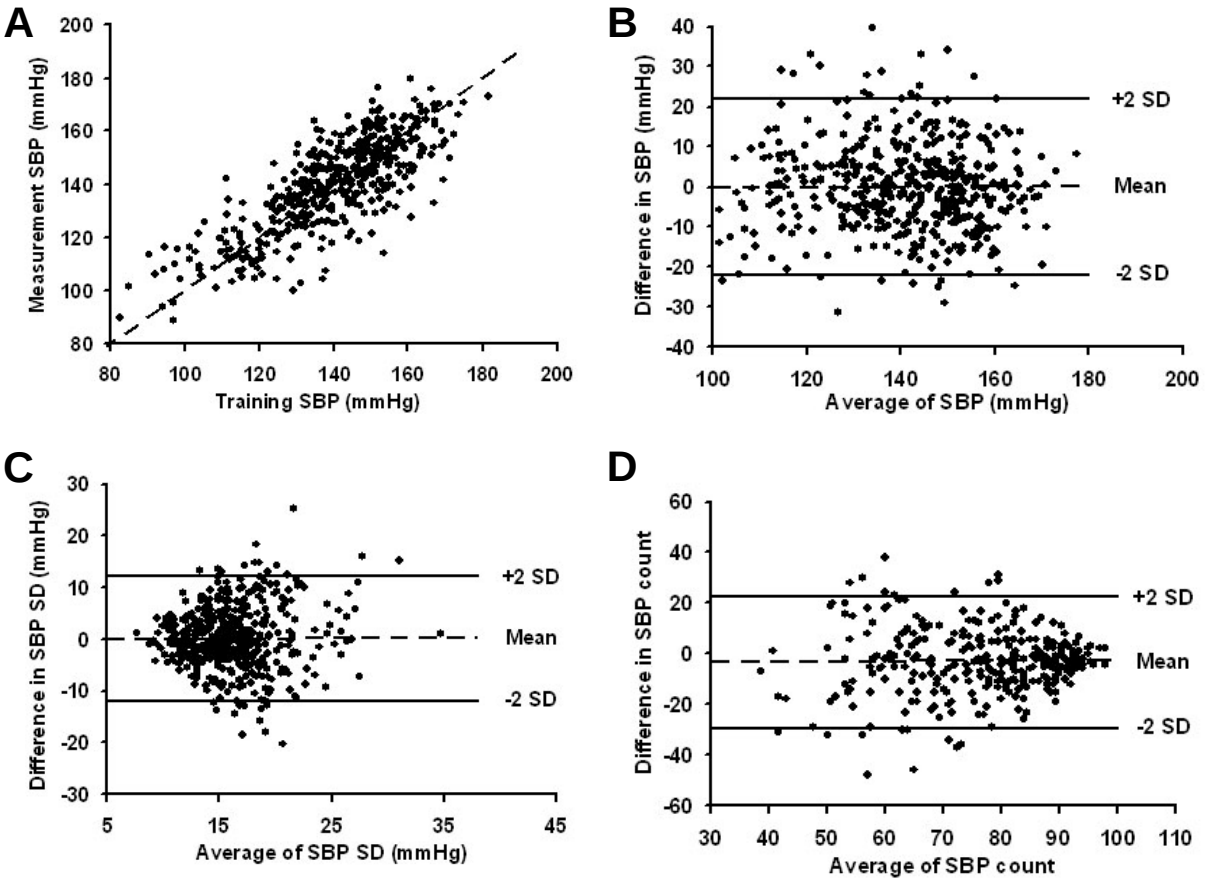

Figure S1: Blood pressure measurement by VPR is not improved by a training week preceding the measurement week. A. Correlation of average systolic blood pressure measurements from the measurement week vs. the training week. If measurements agreed perfectly, all points would fall on the line of identity (the diagonal dash line). Bland-Altman analyses (training week minus measurement week) of systolic blood pressure (SBP; **B**), standard deviation of systolic blood pressure (SBP SD; **C**), and systolic blood pressure count (SBP count; **D**) indicate that the training week measurements do not differ from measurement week measurements. The mean line represents the average difference between the training and measurement weeks and the SD lines reflect two standard deviations from the mean.

Table S1: Summary of Blood Pressure Differences Between Mice from 37 Inbred Strains

	n	Mean	SD																																
C3H/HeJ	11	100.5	3.2	A																															
SJL/J	11	100.6	4.4	A	B																														
BTBR+ tf/J	10	101.4	2.7	A	B	C																													
LP/J	10	102.6	7.1	A	B	C	D																												
C57L/J	12	103.1	3.1	A	B	C	D	E																											
C57BL/10J	11	103.4	2.8	A	B	C	D	E	F																										
DBA/1J	10	103.4	2.5	A	B	C	D	E	F	G																									
C57BR/cdJ	10	104.8	4.9	A	B	C	D	E	F	G	H																								
NON/ShiLtJ	10	104.8	6.1	A	B	C	D	E	F	G	H	I																							
CBA/J	10	105.5	7.1	A	B	C	D	E	F	G	H	I	J																						
BALB/cJ	10	105.9	3.3	A	B	C	D	E	F	G	H	I	J	K																					
A/J	13	106.5	5.6	A	B	C	D	E	F	G	H	I	J	K	L																				
C58/J	11	106.8	2.6	A	B	C	D	E	F	G	H	I	J	K	L	M																			
NZW/LacJ	9	107.0	8.3	A	B	C	D	E	F	G	H	I	J	K	L	M	N																		
BALB/cAn	10	107.8	3.6	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O																	
CBA/CaJ	9	107.8	3.3	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P																
DBA/2J	10	107.8	3.5	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q															
I/LnJ	6	107.8	6.2	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R														
FVB/N	20	110.0	4.9				D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S													
SM/J	9	110.7	4.6				D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T												
FGS/Nag	5	111.8	6.3			C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U											
KK/Ta	6	111.8	4.1				D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V										
MRL/MpJ	10	113.6	6.5										J	K	L	M	N	O	P	Q	R	S	T	U	V	W									
129S1/SvImJ	10	113.8	6.1										J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X								
C57BL/6J	10	114.6	5.3												L	M	N	O	P	Q	R	S	T	U	V	W	X	Y							
RIIIS/J	10	115.3	6.6														N	O	P	Q	R	S	T	U	V	W	X	Y	Z						
C57BKS/J	10	115.4	3.0														N	O	P	Q	R	S	T	U	V	W	X	Y	Z	a					
129/+Te	10	115.7	8.7														N	O	P	Q	R	S	T	U	V	W	X	Y	Z	a	b				
BUB/BnJ	9	116.0	3.9														N	O	P	Q	R	S	T	U	V	W	X	Y	Z	a	b	c			
PL/J	6	118.8	7.4																		R	S	T	U	V	W	X	Y	Z	a	b	c	d		
NZB/BINJ	12	120.2	4.0																					U	V	W	X	Y	Z	a	b	c	d	e	
ALS/LtJ	7	120.6	6.6																					U	V	W	X	Y	Z	a	b	c	d	e	f
AKR/J	9	121.2	3.1																					U	V	W	X	Y	Z	a	b	c	d	e	f	g
NOD/Shi	12	127.1	4.4																														d	e	f	g	h
SWR/J	9	127.1	3.0																														d	e	f	g	h
BPH/2J	10	131.7	3.9																																		h
NZO/H1LtJ	12	132.4	3.1																																		h

Blood pressure is not significantly different between strains sharing one or more letters. For example, mice from any strain with J – W in its row (indicated by the gray box) are not significantly different from MRL/MpJ mice. Differences were determined by TukeyHSD test.

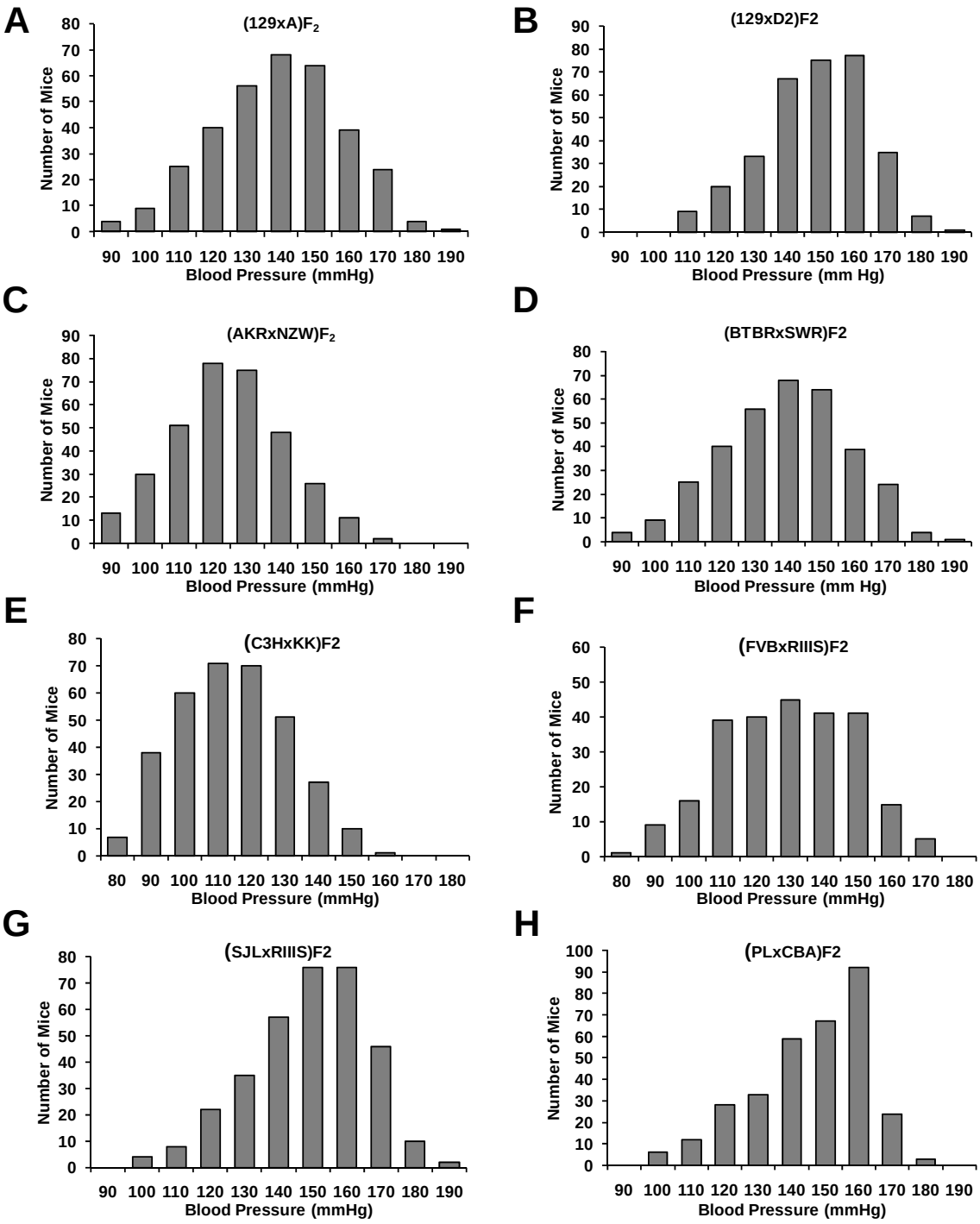

Figure S2: Distribution of blood pressure in eight intercross populations.

Table S2. Significant QTL for kidney weight, with body weight as a covariate, identified in the eight intercrosses.

Chr	Cross	Peak (cM)	95% CI (cM)	95% CI (Mb)	LOD	High Allele
1	BTBRxSWR	30.6	19.8 - 60.6	38.94 - 122.35	3.57	SWR
	129xD2	45.6	25.0 - 64.9	49.44 - 130.73	6.11	129
3	SJLxRIII	37.8	19.6 - 57.7	39.42 - 115.47	5.19	SJL
	PLxCBA	49.2	32.9 - 65.6	65.42 - 130.72	4.02	PL
4	C3HxKK	51.6	44.5 - 58.6	90.84 - 118.83	11.9	C3H
	129xAJ	62.0	46.6 - 73.6	94.98 - 150.56	3.93	AJ
	129xD2	62.0	17.1 - 62.0	34.65 - 126.21	3.74	D2
5	BTBRxSWR	25.6	1.6 - 40.1	10.68 - 81.67	4.64	SWR
	129xD2	54.5	39.1 - 67.4	79.69 - 136.54	11.20	D2
6	FVBxRIII	33.1	5.1 - 57.1	10.33 - 113.29	3.49	FVB
	SJLxRIII	33.5	16.5 - 57.6	32.77 - 114.21	4.90	RIII
10	SJLxRIII	42.6	35.2 - 49.8	69.80 - 98.88	9.50	RIII
	129xD2	52.2	22.5 - 60.1	44.66 - 119.47	3.61	D2
11	AKRxNZW	12.1	6.1 - 32.7	12.19 - 64.68	4.03	AKR
	FVBxRIII	26.2	14.6 - 48.6	29.13 - 96.34	3.49	FVB
12	PLxCBA	17.2	3.2 - 35.3	6.56 - 76.92	3.53	PL
14	C3HxKK	45.5	27.6 - 56.6	58.86 - 120.97	6.14	C3H
15	BTBRxSWR	41.1	24.6 - 51.4	44.66 - 119.47	3.93	BTBR
17	AKRxNZW	13.9	9.9 - 40.1	20.43 - 80.87	3.59	NZW
19	SJLxRIII	13.2	3.2 - 29.1	4.43 - 58.18	4.46	RIII

QTL, quantitative trait locus; Chr, chromosome; CI, confidence interval; cM, centimorgan; Mb, megabase; LOD, logarithm of the odds ratio.