Online Supplement:

Genetic analysis of blood pressure in eight mouse intercross populations

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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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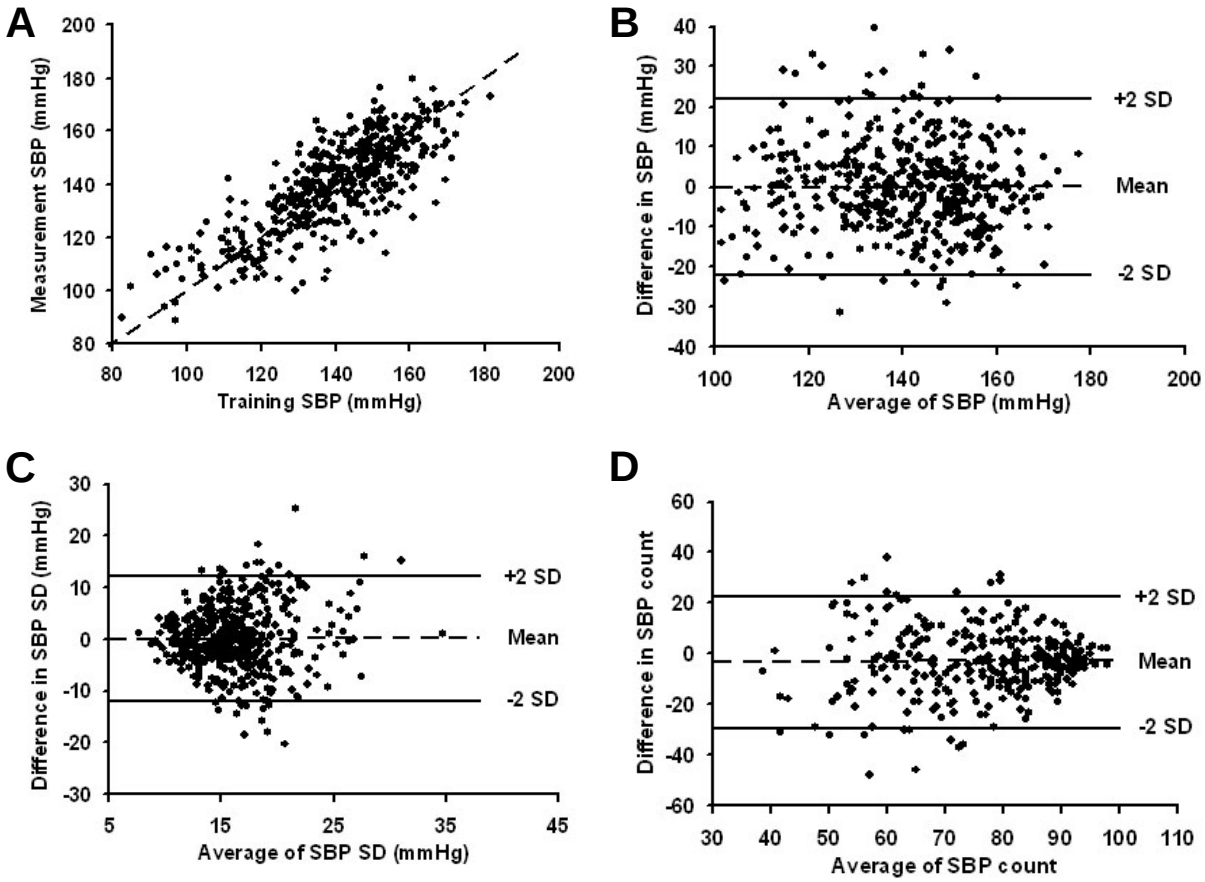

Figure S1: Blood pressure measurement by VPR is not improved by a training week preceding the measurement week. A. Correlation of average systolic blood pressure measurements from the measurement week vs. the training week. If measurements agreed perfectly, all points would fall on the line of identity (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.

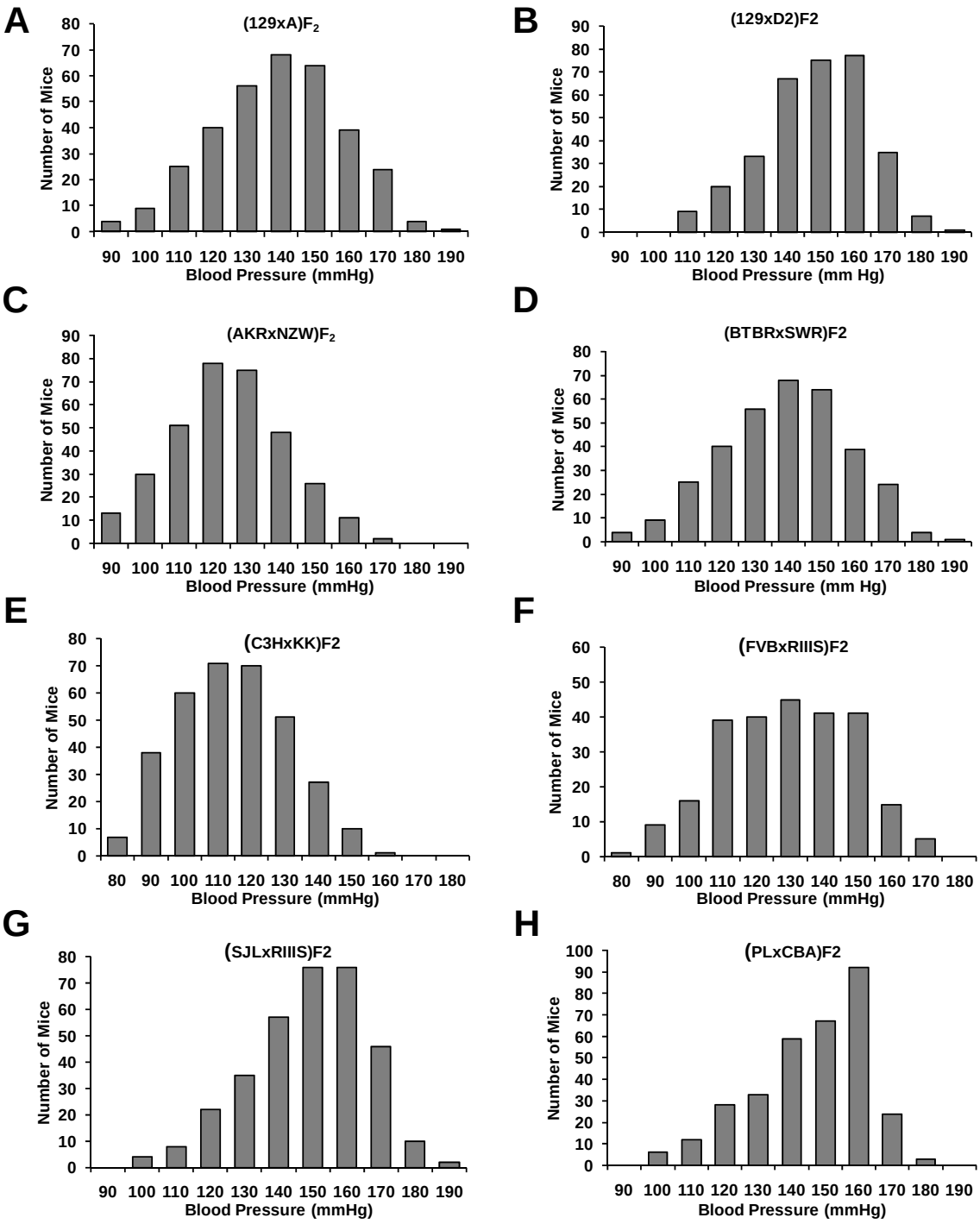

Figure S2: Distribution of blood pressure in eight intercross populations.

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.