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Gender-Specific Genetic Determinants of Blood Pressure and Organ Weight: Pharmacogenetic Approach

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Summary

A total genome scan and pharmacogenetic study were designed to search for genetic determinants of blood pressure (BP) as well as heart and kidney weights. Genome scanning was carried out in 266 F_2 intercrosses from Prague hypertensive hypertriglyceridemic rats for phenotypes of organ weights, baseline BP, BP after blockade of the renin-angiotensin system (RAS) by losartan, of the sympathetic nervous system (SNS) by pentolinium, and of the nitric oxide (NO) synthase by N^G-nitro-L-arginine methyl ester. Pharmacogenetic analysis showed that, in males, BP was controlled by two loci on chromosomes 1 and 5 (Chr1, Chr5) through the SNS, and these loci showed a positive contribution for relative kidney weight (KW/BW). On the other hand, baseline BP in females was controlled by two loci on Chr3 and Chr7. The effect of these loci was not mediated by the RAS, SNS or NO system. These loci did not show any effect for KW/BW. Negatively-linked loci for KW/BW and relative heart weight (HW/BW) were identified on Chr2 in both genders. Another negatively-linked locus for KW/BW, located on Chr8 in males, affected BP through the SNS. This locus on Chr8 overlapped with a previously-reported modifier locus for polycystic kidney disease (PKD). In conclusion, this pharmacogenetic study determined two loci for BP and relative organ mass implicating sympathetic overactivity. Concordance of the identified locus for KW/BW and BP through the SNS on Chr8 with the PKD locus revealed the importance of this region for renal complications in various diseases.

Key words

Prague hypertriglyceridemic rat • Quantitative trait locus • Heart weight • Kidney weight • Hypertension • Pharmacogenetics

Introduction

Hypertension affects about one-quarter of adults in industrialized countries (Joffres *et al.* 2001) and contributes to morbidity and mortality from heart failure, coronary heart disease, stroke and renal failure (Devereux *et al.* 1994, Levy *et al.* 1990). Cardiac hypertrophy, particularly left ventricular hypertrophy, carries a significant risk for cardiovascular diseases (Kannel *et al.* 1970) with its own familial and presumable genetic risk

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factors (Verhaaren *et al*. 1991). The susceptibility of hypertensive patients to end-stage renal disease seems to vary with ethnicity (Freedman *et al*. 1993, Excerpts from the United States Renal Data System 1996). In contrast to the clear link between malignant hypertension and renal failure (Brazy *et al*. 1989, Freedman *et al*. 1995, Klahr *et al*. 1988), only a small percentage of patients with mild to moderate hypertension develop renal failure (Perneger *et al*. 1993).

In animal models of hypertension, susceptibility to renal damage and increased cardiac mass has been attributed to genetic factors (Weening *et al*. 1986, Brown *et al*. 1996). A positive correlation between systolic blood pressure (SBP) and relative heart weight (HW/BW) was found, while relative kidney weight (KW/BW) correlated negatively with blood pressure (BP) in recombinant inbred strains derived from spontaneously hypertensive rats (SHR) and Brown Norway (BN) rats (Kuneš *et al.* 1990). On the other hand, other genetic determinants of heart weight can act independently of BP (Hamet *et al*. 1996a, 1998). Folkow (1982) developed the notion that cardiovascular system proliferation may be an important component in the pathogenesis of hypertension. We proposed earlier that a proliferative process might be primarily involved in hypertension development because it is present at birth and persists in cultured cells of genetically hypertensive rats (Hadrava *et al*. 1989, Hamet *et al*. 1985). More recently, we added the notion of imbalance between proliferation and apoptosis, which is apparently accentuated in the later stages of hypertension (Hamet *et al*. 1995, 1996b, 2001) and may even be a part of the pathogenetic process.

The Prague hypertensive hypertriglyceridemic (HTG) rat is a novel strain that develops several major features of syndrome X, such as hypertension, hypertriglyceridemia, hyperinsulinemia and impaired glucose tolerance (Štolba *et al*. 1993, Vrána *et al*. 1993). In addition to hypertension, hyperlipidemia and hyperinsulinemia have been shown to accelerate cell proliferation (Nishida *et al*. 1999, Absher *et al*. 1999). Thus, it is important to identify the genetic components of organ weight in this strain and to understand the pathogenetic mechanisms of organ weight determination. We have recently established a new approach to estimate the contribution of BP regulatory systems, using a pharmacogenetic approach (Ueno *et al*. 2003a). The current study was designed to perform a total genome scan and pharmacogenetic analysis of F_2 crosses between HTG and normotensive Lewis rats. Consequently, quantitative trait loci (QTLs) for phenotypes such as KW/BW, HW/BW and BP changes during sequential pharmacological subtraction of BP were searched to investigate the genetic mechanisms controlling BP and relative organ weights.

Methods

Animal procedure

HTG rats were derived from a colony of Wistar rats (Vrána and Kazdová 1990), and their characteristics have been described previously (Štolba *et al.* 1992, 1993). Normotensive Lewis and hypertensive HTG rats (Institute of Physiology AS CR, Prague) were reciprocally mated to produce F_1 hybrids. Females and males of the F_1 generation were randomly mated to provide F_2 cohorts for the examination of several cardiovascular phenotypes (Kuneš *et al.* 2002). The procedures and experimental protocols were approved by the local ethics committee of the Institute of Physiology AS CR, Prague. Progenitors and 266 F_2 hybrids (137 males and 129 females) were phenotyped at the age of 5-6 months.

BP recording

Under light ether anesthesia, polyethylene catheters were inserted into the left carotid artery and jugular vein, and BP was recorded in conscious animals after 24-h recovery. Basal mean arterial blood pressure (MAP) values were monitored for 30 min. Thereafter, an intravenous bolus of losartan (Du Pont-Merck, Wilmington, 10 mg/kg BW) was injected to block angiotensin AT_1 receptors. Ten minutes later, ganglion blockade was induced by pentolinium injection (Sigma, St. Louis, 5 mg/kg BW). When low residual BP values were stabilized (usually after 5 min), N^G-nitro-L-arginine methyl ester (L-NAME) (Sigma, 30 mg/kg BW) was given to block nitric oxide synthases, and BP restoration was monitored for at least 20 min. All drugs were delivered intravenously in a volume of 1 ml/kg BW. At the end of the experimental protocol, the animals were sacrificed by decapitation, their heart and kidneys were weighed, and their livers were excised and frozen in liquid nitrogen. Genomic DNA was extracted from this organ by a standard phenol-chloroform method.

Genome scan

135 single strand length polymorphism (SSLP) markers covering 21 chromosomes (autosomes and X) were selected to provide a genomic map with an inter-marker average distance of approximately 20 cM. This

distance was expected to limit a minimal number of false negative results, as previous theoretical computations have suggested (Almasy and Blangero 1998, Darvasi and Soller 1995). To increase the efficacy of this step, an approach was adopted to select animals with extreme phenotypes; 46 progeny that represented the highest and lowest phenotypic extremes for MAP, KW/BW, HW/BW and BW traits were chosen. Our approach maximized genetic contrast and potential linkage information. The complete genome-wide study of this group allowed us to build a genetic linkage map for our cross, and provided preliminary mapping information on loci for MAP with greater efficiency than scanning of all F_2 rats (Darvasi and Soller 1992, Darvasi 1997). Chromosomal regions of interest were identified on the basis of a logarithm of likelihood (LOD) >1.0. For the regions of interest, the density of the markers was augmented to reach an inter-marker distance of less than 10 cM, and the number of genotyped animals was also increased to include all 266 F_2 hybrids. Thus, we genotyped all 266 F_2 rats with high-density markers on chromosomes (Chr) 1, 2, 3, 5, 7 and 8. SSLP marker information and mapping data were taken from: Whitehead Institute/Massachusetts Institute of Technology rat database (Cambridge, MA; http: //waldo.wi.mit.edu/rat/public), the Wellcome Trust Centre for Human Genetics (Oxford, UK; http: //www.well.ox.ac.uk), and The Journal of Clinical Investigation (Deng *et al.* 1994). In the second scanning step, 56 additional markers were subsequently typed in suggestive loci identified in the initial genome-wide scan. At each locus, peak markers and additional closely-linked markers were typed in the entire set of rats. Finally, 191 markers covered the genetic map length of 1,634.9 cM, and the averaged genetic distance between adjacent markers was 10.5 cM. The average distance of markers in the regions of interest was 6.1 cM.

Statistical analysis

The normality of all phenotypes was examined by application of the Kolmogorov-Smirnov test. Phenotypes that did not pass the normality test were then corrected by log transformation. The significance of differences within and between groups was determined by one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test. Construction of linkage maps and QTL mapping were performed by the Map Manager QT program (Version 3.0b, Manly and Olson 1999). The significance of each potential association was measured by likelihood ratio statistics (LRS). Then, the LRS were converted to conventional base-10 LOD scores by division with 4.61. The free genetic model was evaluated for each of the traits reported here. A permutation test, by randomly assigning the phenotypes relative to the genotypes in 1,000 replicated tests, was used to determine the threshold of significance in this free genetic model.

Results

Clinical characteristics of parental strains and F_2 populations

In progenitor strains, SBP, diastolic blood pressure (DBP) and HW/BW were significantly higher ($p<0.05$) in hypertensive HTG than in normotensive Lewis rats. In contrast, KW/BW was significantly lower in HTG rats (Table 1). There were gender differences among phenotypes studied in F_2 hybrids. Relative HW/BW and SBP were lower, whereas DBP was higher in males compared to females.

Table 1. Body weight (BW), relative heart weight (HW/BW), relative kidney weight (KW/BW), systolic blood pressure (SBP) and diastolic blood pressure (DBP) in progenitor strains and F_2 hybrids.

	n	BW (g)	HW/BW (g/100 g)	KW/BW (g/100 g)	SBP (mm Hg)	DBP (mm Hg)
HTG	10	309.3 ±15.9	0.267 ±0.002*$^+$	0.592 ±0.005*$^+$	163.4 ±4.8*$^+$	108.8 ±4.3*$^+$
LEW	9	300.2 ±8.0	0.250 ±0.005$^+$	0.684 ±0.011$^+$	128.5 ±3.5	82.5 ±3.1
F_2 rats	266	301.8 ±4.3	0.231 ±0.001	0.519 ±0.003	135.1 ±0.6	90.5 ±0.6
F_2 males	137	362.9 ±3.4‡	0.218 ±0.001‡	0.521 ±0.004	133.8 ±0.8‡	92.8 ±0.8‡
F_2 females	129	238.7 ±1.8	0.246 ±0.001	0.516 ±0.004	136.5 ±1.0	88.2 ±0.8

** $p<0.05$ vs Lewis rats, $^+$ $p<0.05$ vs F_2, ‡ $p<0.05$ versus F_2 females*

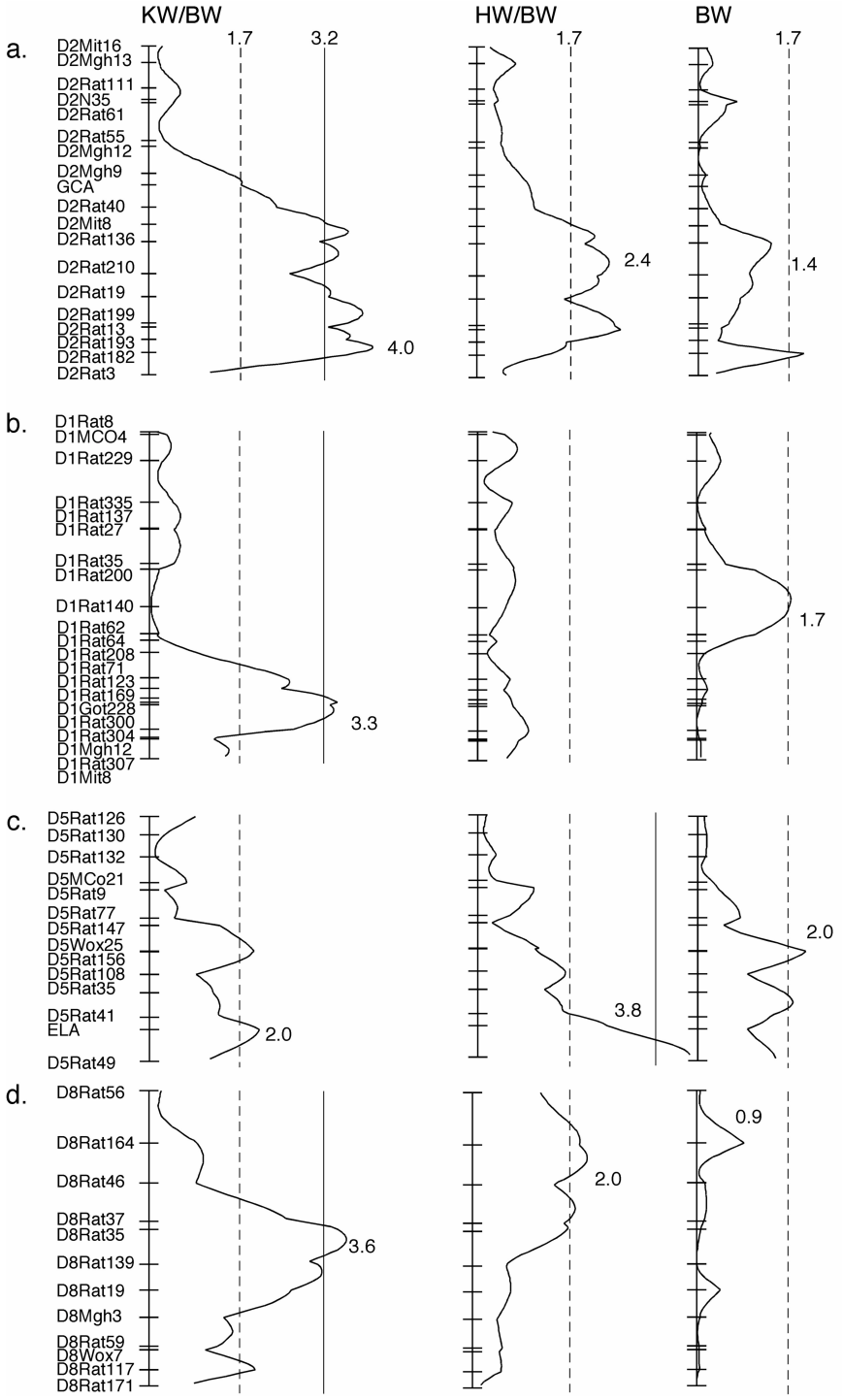

Fig. 1. *QTL plots for relative kidney weight (KW/BW), relative heart weight (HW/BW) and body weight (BW) on chromosomes 2, 1, 5 and 8 in male rats. The significance of each potential association is measured by a logarithm of likelihood (LOD). The dashed line is the LOD threshold for suggestive linkage; the continuous line is for significant linkage calculated by the permutation test.*

Males

Figure 1 shows the results of multi-locus linkage analysis in chromosomal regions corresponding to the effects that were above suggestive on permutation testing for KW/BW, HW/BW and BW in males. The strongest evidence of a QTL was found in the broad region on rat Chr2 for both KW/BW and HW/BW with a negative correlation. Maximal effects were noted between D2Rat182 and D2Rat40 for KW/BW (LOD 4.0) (Fig. 1a), whereas no region was identified as the significant locus for BP on Chr2. On Chr1, LOD reached 3.3 for KW/BW in the region between D1Rat71 and D1Mgh12 with a positive contribution (Fig. 1b). This region showed linkage for baseline MAP (LOD 3.2), and the linkage was only partially affected by blockade of the RAS by losartan (LOD 2.0), but disappeared after blockade of the SNS by pentolinium (LOD 0.7). Interestingly, another linkage appeared after the

inhibition of NO synthase by L-NAME (LOD 3.8) around D1Rat123 (Fig. 2a). A suggestive positive QTL was found for KW/BW (between D5Rat77 and D5Rat108, LOD 1.8) with a positive correlation on rat Chr5 (Fig. 1c). This QTL for KW/BW overlapped with suggestive QTL for MAP (between D5Rat77 and D5Rat108, LOD 2.9), and was partially affected by blockade of the RAS (LOD 1.8), but was completely attenuated after blockade of the SNS by pentolinium (LOD 0.1). Negative QTLs for both KW/BW and HW/BW were detected between D8Rat164 and D8Mgh3 on Chr8 (LOD 3.6) (Fig. 1d). In this region, no significant linkage was localized for baseline MAP or MAP after losartan, whereas negative correlation was localized for MAP after blockade of the SNS by pentolinium (LOD 3.0) (Fig. 2c). The results of one-way ANOVA were consistent with data obtained by the Map Manager QT program (Tables 2 and 3).

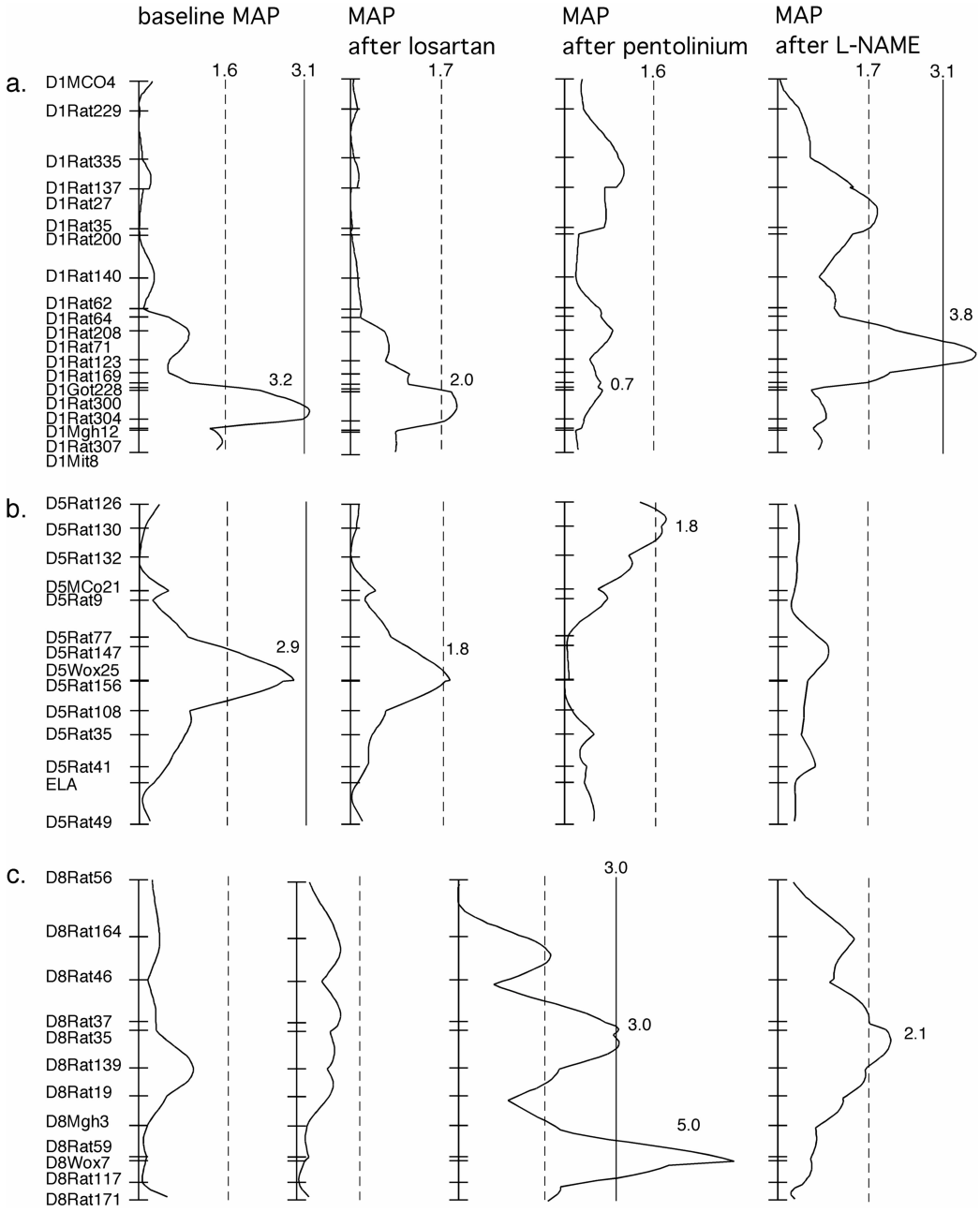

Fig. 2. *QTL plots for blood pressure achieved during the sequential blockade of blood pressure regulatory systems on chromosomes 1, 5 and 8 in male population. The significance of each potential association is measured by a logarithm of likelihood (LOD). The dashed line is the LOD threshold for suggestive linkage; the continuous line is for significant linkage calculated by the permutation test.*

Table 2. Total genome search: significant and suggestive quantitative trait loci for relative kidney weight (KW/BW), relative heart weight (HW/BW) and body weight (BW) in F_2 rats of both genders.

Locus	LOD	%	P	HTG/HTG	HTG/LEW	LEW/LEW
	F_2 males					
	KW/BW					
D1Got228	3.32	9	<0.001	0.537 ±0.005*	0.521 ±0.005*	0.498 ±0.008
D2Rat199	3.88	11	<0.001	0.502 ±0.006*,**	0.526 ±0.005	0.542 ±0.008
D2Rat182	4.03	11	<0.001	0.496 ±0.007*,**	0.529 ±0.005	0.531 ±0.007
D5Wox25	1.87	5	0.013	0.542 ±0.007*,**	0.518 ±0.005	0.513 ±0.006
D8Rat35	3.69	10	<0.001	0.503 ±0.007*	0.520 ±0.005*	0.545 ±0.006
	HW/BW					
D2Rat210	2.00	5	0.011	0.212 ±0.002**	0.220 ±0.002	0.220 ±0.002
D2Rat199	2.26	6	0.005	0.212 ±0.002*,**	0.220 ±0.002	0.221 ±0.003
D8Rat164	1.89	5	0.013	0.210 ±0.003*	0.218 ±0.002	0.221 ±0.002
	BW					
D1Rat140	1.67	4	0.022	353.3 ±6.1*	360.1 ±4.3	379.0 ±7.9
	F_2 females					
	KW/BW					
D2Rat19	5.77	17	<0.001	0.497 ±0.100*	0.511 ±0.004*	0.549 ±0.007
D2Rat182	5.42	16	<0.001	0.490 ±0.009*,**	0.518 ±0.004*	0.542 ±0.009
	HW/BW					
D2Rat136	2.73	8	0.002	0.237 ±0.002*,**	0.246 ±0.002	0.250 ±0.003
D2Rat199	2.82	8	0.001	0.239 ±0.002*	0.245 ±0.007*	0.584 ±0.008
	BW					
D2Mgh9	4.88	15	<0.001	379.0 ±7.9*,**	235.1 ±2.6	232.4 ±2.7
D2Mit8	3.36	0	<0.001	251.8 ±4.4*,**	239.3 ±2.7	232.3 ±2.5
D2Rat13	2.39	7	0.004	246.8 ±3.1*	240.3 ±2.7*	229.4 ±2.9
D8Rat139	2.95	9	0.001	229.6 ±3.6*	238.9 ±2.4	248.5 ±3.6

** p<0.05 vs LEW/LEW, ** p<0.05 vs HTG/LEW by Tukey-Kramer test*

Females

In females, maximal effects for both KW/BW and HW/BW were noted between D2Rat40 and D2Rat182 (LOD 6.6 and 3.0) with negative correlations, whereas no region was located as a significant locus for BP on Chr2 (Fig. 3a). Regions between D2Rat61 and D2Rat40 on Chr2 and between D8Rat37 and D8Rat59 on Chr8 showed significant linkage with BW in this gender (LOD 5.0 and 3.1) (Fig. 3a and 3b). A QTL located between markers D3Rat54 and D3Rat17 on Chr3 revealed significant linkage with baseline MAP (LOD 4.6) (Fig. 4a). This QTL was not affected by blockade of the RAS with losartan (LOD 3.8). Linkage of the locus with MAP remained suggestive after blockade of the SNS by pentolinium (LOD 2. 0), and after inhibition of the NO system by L-NAME (LOD 2.1) (Fig. 4a). On Chr7, a locus between D7Rat141 and D7Rat115 showed suggestive linkage with baseline MAP, and this QTL remained significant after the administration of losartan (LOD 2.4), pentolinium (LOD 3.8) and L-NAME (LOD 2.1) (Fig. 4b).

Table 3. Significant and suggestive quantitative trait loci for mean arterial pressure during pharmacogenetic analysis in F_2 rats of both genders.

Locus		LOD	%	P	HTG/HTG	HTG/LEW	LEW/LEW
	F_2 males						
D1Rat304	baseline MAP	3.06	8	0.001	117.2 ±0.8**	110.7 ±1.7	113.5 ±1.7
	$MAP_{losartan}$	1.74	4	0.018	113.0 ±1.4**	107.4 ±1.3	109.1 ±1.9
	$MAP_{pentolinium}$	0.22	0	0.604	47.9 ±1.4	46.4 ±0.9	47.5 ±1.1
D1Rat71	$MAP_{L\text{-}NAME}$	3.67	10	<0.001	129.6 ±2.4*,**	116.2 ±2.0	118.2 ±3.6
D5Wox25	baseline MAP	2.86	8	0.001	118.1 ±1.6*,**	113.0 ±1.1	110.2 ±1.2
	$MAP_{losartan}$	1.80	5	0.019	113.9 ±2.0*	109.2 ±1.2	106.6 ±1.5
	$MAP_{pentolinium}$	0.09	0	0.833	47.2 ±1.3	47.3 ±0.9	46.6 ±1.3
	$MAP_{L\text{-}NAME}$	0.48	0	0.336	124.9 ±3.1	120.0 ±2.3	118.8 ±2.5
D8Rat35	baseline MAP	0.30	0	0.050	111.6 ±1.8	113.8 ±1.1	113.1 ±1.4
	$MAP_{losartan}$	0.56	0	0.268	106.8 ±2.0	110.1 ±1.5	110.3 ±1.5
	$MAP_{pentolinium}$	2.89	8	0.001	43.1 ±1.1*,**	48.1 ±0.9	48.6 ±1.3
	$MAP_{L\text{-}NAME}$	2.04	5	0.009	117.0 ±3.6*	118.9 ±1.9*	129.4 ±2.9
D8Rat59	baseline MAP	0.11	0	0.788	113.3 ±0.8	112.7 ±1.1	114.0 ±1.5
	$MAP_{losartan}$	0.20	0	0.645	109.2 ±2.1	109.0 ±1.2	110.6 ±1.5
	$MAP_{pentolinium}$	5.01	14	<0.001	44.9 ±1.0*	45.4 ±0.8*	51.8 ±1.4
	$MAP_{L\text{-}NAME}$	0.54	0	0.289	121.3 ±3.0	118.6 ±2.2	123.8 ±2.7
	F_2 females						
D3Mgh6	baseline MAP	4.43	13	<0.001	115.0 ±1.5*	113.3 ±1.2*	105.9 ±1.5
	$MAP_{losartan}$	3.54	10	<0.001	109.4 ±1.7*	107.6 ±1.3*	100.1 ±1.8
	$MAP_{pentolinium}$	1.76	5	0.018	44.0 ±1.1	44.6 ±0.8*	41.1 ±1.0
	$MAP_{L\text{-}NAME}$	2.06	6	0.009	112.1 ±3.1	113.4 ±2.6*	101.3 ±3.2
D7Rat135	baseline MAP	1.76	5	0.017	115.7 ±1.8*	111.0 ±1.1	109.1 ±1.7
	$MAP_{losartan}$	2.21	6	0.006	111.1 ±2.1*	105.0 ±1.2	102.9 ±2.0
	$MAP_{pentolinium}$	1.89	5	0.013	46.1 ±1.2*,**	43.0 ±0.8	41.7 ±0.9
	$MAP_{L\text{-}NAME}$	0.76	1	0.176	115.0 ±3.2	108.1 ±2.5	107.7 ±3.6
D7Rat5	baseline MAP	1.65	4	0.022	114.3 ±1.6*	112.3 ±1.2	107.9 ±1.7
	$MAP_{losartan}$	2.43	7	0.004	110.2 ±1.8*	106.3 ±1.3	101.2 ±1.9
	$MAP_{pentolinium}$	2.89	8	0.001	46.8 ±1.2*,**	42.9 ±0.7	41.2 ±0.9
	$MAP_{L\text{-}NAME}$	1.69	4	0.021	117.1 ±3.3*	108.8 ±2.3	104.0 ±3.9

** $p<0.05$ vs LEW/LEW,** $p<0.05$ vs HTG/LEW by Tukey-Kramer test, $MAP_{losartan}$ - MAP after losartan, $MAP_{pentolinium}$ - MAP after pentolinium, $MAP_{L\text{-}NAME}$ - MAP after L-NAME administration.*

Discussion

We have previously reported the establishment of methods to evaluate the contribution of BP regulatory systems for BP maintenance by a pharmacogenetic approach (Ueno *et al*. 2003a). In this analysis, the disappearance of a QTL for MAP after BP regulatory system blockade reveals the contribution of the blocked system for baseline MAP, whereas the absence of the blockade effect indicates a negligible contribution of the blocked system. Using this approach, we determined that baseline BP of the male population was controlled by two loci on Chr1 and Chr5 through the SNS. On the other hand, baseline BP of our female population was

controlled by two loci on Chr3 and Chr7, which were independent of the RAS, SNA and NO system. Although these male and female populations were derived from the same progenitors, their BP was controlled by distinct loci through different BP regulatory systems. While sexual specificity of the QTL effects can be explained by a sex chromosome action, hormonal interaction at the transcriptional and post-transcriptional levels represents an alternative possibility that needs to be addressed in future studies. Age at phenotyping is another important issue to be addressed. In this study, we were evaluating phenotypes only at one point in life for each rat (20-24 weeks of age). It should be noted that Kovács *et al*. (1998) reported age-dependent changes of the effects exerted by "lipid loci". For example, a QTL for triglycerides had maximal impact at 20 weeks of age, but its influence disappeared at the age of 32 weeks.

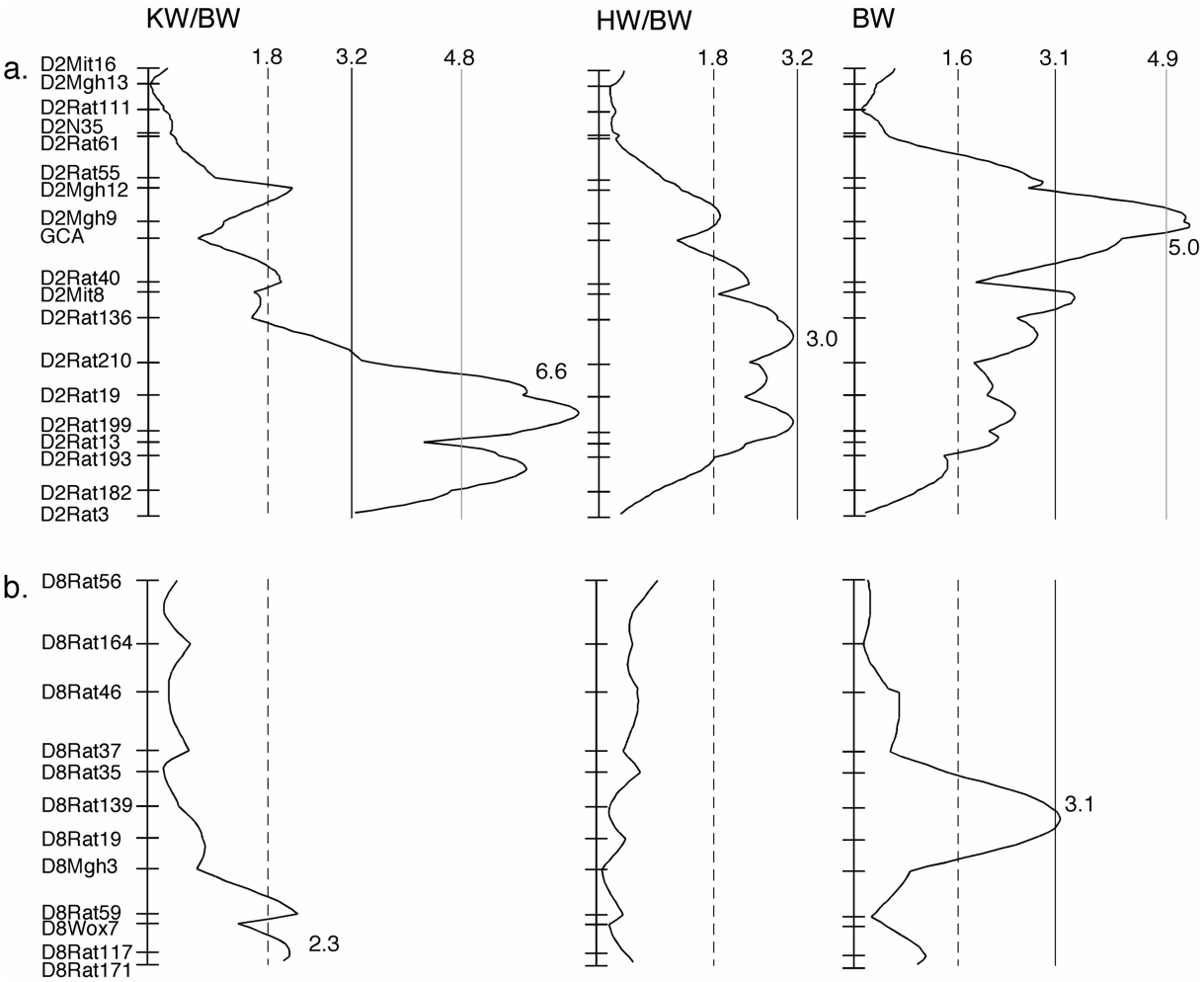

Fig. 3. *QTL plots for relative kidney weight (KW/BW), relative heart weight (HW/BW) and body weight (BW) on chromosomes 2 and 8 in female population. The significance of each potential association is measured by a logarithm of likelihood (LOD). The dashed line is the LOD threshold for suggestive linkage; the continuous black line is for significant linkage; the continuous gray line is for highly significant linkage calculated by the permutation test.*

In males, KW/BW was controlled by the balance of two positively-correlated loci (Chr1 and Chr5) and two negatively-correlated loci (Chr2 and Chr8). These positive loci for KW/BW on Chr1 and Chr5 overlapped with QTL for baseline MAP with a suggestive level of correlation. By the pharmacogenetic approach, two loci on Chr1 and Chr5 affected baseline BP through the SNS in males, whereas two loci for baseline BP on Chr3 and Chr7 affected baseline MAP without any contribution of examined BP regulatory systems. These two loci did not

show any association with KW/BW in females. Increased sympathetic tone in patients with end-stage renal failure has been reported (Converse *et al*. 1992), and the kidney might be an origin as well as a target of SNS overactivity (Campese 1997). Rump *et al*. (2000) documented a possible effect of sympathetic overactivity in end-stage renal failure, but they mentioned that it was too early to generally recommend sympatholytic drugs for patients with chronic renal failure. Applying the pharmacogenetic approach to hypertensives will be a new tool to select patients for whom sympatholytic drugs may reduce the risk of renal complications.

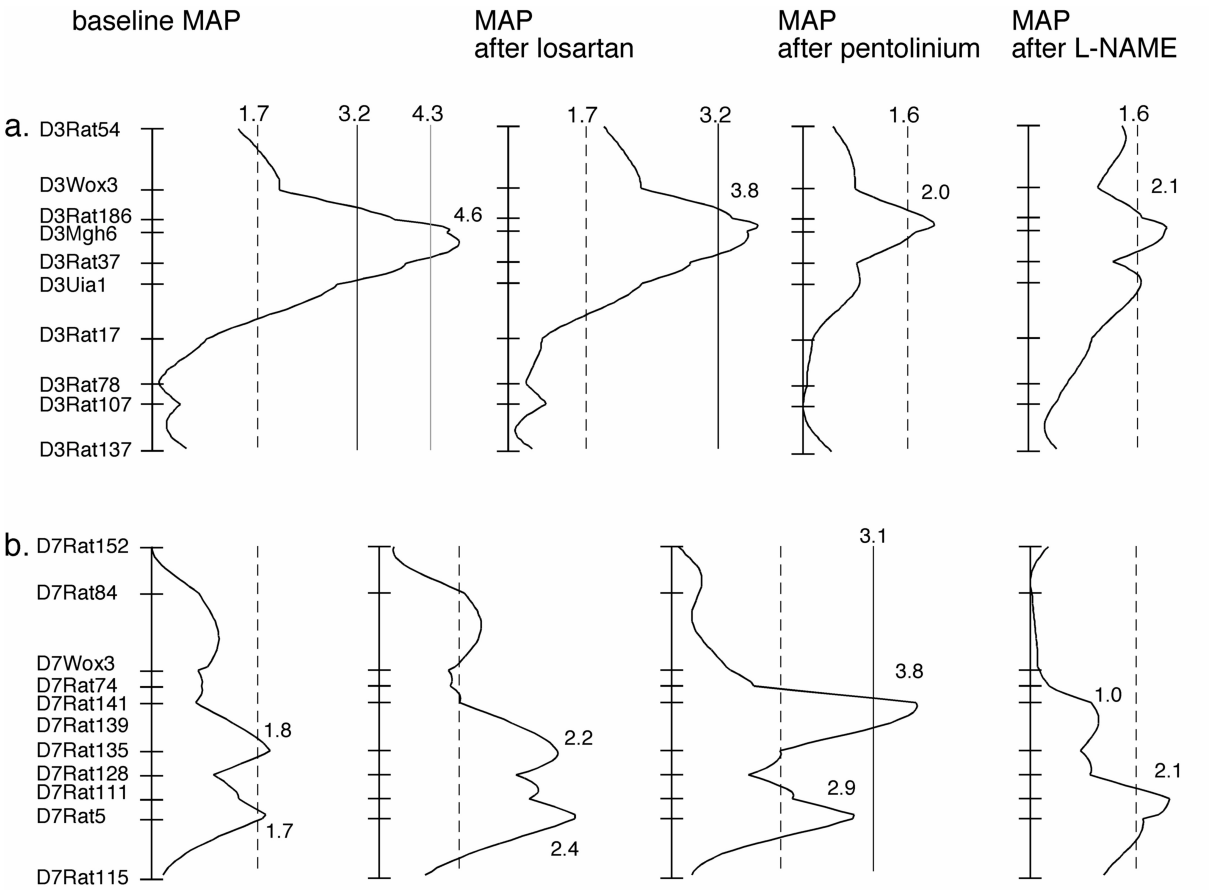

Fig. 4. *QTL plots for blood pressure achieved during the sequential blockade of blood pressure regulatory systems on chromosomes 3 and 7 in female population. The significance of each potential association is measured by a logarithm of likelihood (LOD). The dashed line is the LOD threshold for suggestive linkage; the continuous line is for significant linkage; the continuous gray line is for highly significant linkage calculated by the permutation test.*

In males, negative QTL for KW/BW was detected between D8Rat164 and D8Mgh3, and showed linkage with MAP which was significant only after SNS blockade with pentolinium (Ueno *et al*. 2003a). This QTL overlapped with a previously-reported modifier locus for polycystic kidney disease (PKD), *Modpkdr*, on rat Chr8 that controls the severity of renal damage, kidney mass and plasma urea concentration (Bihoreau *et al*. 2002). QTL concordance for KW/BW in our novel hypertensive strain of rats with the *Modpkdr* locus in the model strain of PKD suggests the importance of this locus for the progression of renal failure in general renal disorders. These data indicate that the locus is an important therapeutic target to control renal disease. Genotyping at this region may enable us to identify patients from the renal disease population, as a target for therapy with SNS blockers to reduce the risk of progression to renal failure.

A maximal effect for KW/BW was noted between D2Rat182 and D2Rat40 with a negative correlation in both genders. This locus also showed suggestive linkage with HW/BW and BW in both genders. We previously reported linkage of the locus with plasma triglyceride level, and overlapping of its syntenic region on human chromosome with a locus for metabolic

syndrome X (Ueno *et al*. 2003b). Taken together, this locus makes some contribution to triglyceride accumulation in plasma as well as organ weight, including the kidney and heart. It has been suggested that one of the primary defects in the pathogenesis of essential hypertension is a growth-promoting process. Increased DNA synthesis was noted in both the heart and kidney, suggesting cellular hyperplasia in hypertensive strains (Walter and Hamet 1986, Kuneš *et al*. 1987) and even a shorter cell half-life (Hamet *et al*. 2001) The HTG rat is a novel strain showing several metabolic abnormalities (Štolba *et al.* 1992, 1993). The associated increase in plasma lipoprotein and insulin levels is reported to be related to cell proliferation (Nishida *et al*. 1999, Absher *et al*. 1999). Alteration of the gene(s) involved in this process may play some role in protecting the heart and kidney from excess proliferation in HTG rats. We have searched rat genome databases and syntenic regions in mouse and human databases to identify potential candidate genes on rat Chr2.

The human syntenic region for identified rat QTLs on this chromosome contained genes for CDC2-associated protein (CKS1), programmed cell death 10 (PCD10) and phospholipase D1 (PLD1). Most cells undergoing programmed cell death require the transcriptional activation of genes that are essential for cell death, and the PCD10 gene belongs to these genes. CKS1 is a CDC2-associated gene, and CDC2 inhibition is known to inhibit apoptosis (Yu *et al*. 1998). PLD1 activity has been implicated in numerous cellular pathways, including signal transduction, membrane trafficking, and mitosis regulation (Venable and Obeid 1999).

Brown *et al*. (1996) reported two gene loci on Chr1 of the fawn-hooded hypertensive (FHH) rat. One was responsible for the renal impairment, and the other for blood pressure. FHH rats show moderate hypertension and develop progressive proteinuria and glomerulosclerosis at a relatively young age, leading to premature death due to end-stage renal disease (Provoost 1994, de Keijzer *et al*. 1989). In contrast, the protective effect of reduced diastolic BP against renal complications of hypertension has been reported in several human studies (Siewert-Delle 1999, Bakris *et al*. 2000). Kuneš *et al*. (1990) showed the negative correlation between KW/BW and BP in recombinant inbred strains derived from SHR and BN rats. We have previously reported that the KW of adult (15-week-old) and newborn SHR was higher than that of control BN rats. However, the KW of SHR will be lower at the end-stage of renal disease (Hamet *et al*. 1998). This complex time course of KW changes in hypertensive rats may be determined at each time point by the combined actions of genes positively and negatively affecting KW, the effect of BP and the influence of environmental factors. Such balance could always be different in every study because of different time points, different progression speeds of renal complications in each strains and different genetic backgrounds.

In conclusion, this pharmacogenetic study successfully determined two loci for BP and relative organ mass which are related to sympathetic overactivity. Concordance of the identified locus for KW/BW and BP through the sympathetic nervous system on Chr8 with the PKD locus revealed the importance of this region for renal complications in various diseases.

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