Original Article

Identification of Quantitative Trait Loci for Cardiac Hypertrophy in Two Different Strains of the Spontaneously Hypertensive Rat

Hyoe INOMATA*[1,2], Takehiro WATANABE*[1,2], Yoko IIZUKA*[3], Yi-Qiang LIANG*[1], Tomoji MASHIMO*[4], Toru NABIKA*[5], Katsumi IKEDA*[6], Kazuyuki YANAI*[1], Takanari GOTODA*[3], Yukio YAMORI*[7], Mitsuaki ISOBE*[2], and Norihiro KATO*[1]

Cardiac hypertrophy and left ventricular hypertrophy are known to be substantially controlled by genetic factors. As an experimental model, we undertook genome-wide screens for cardiac mass in F_2 populations bred from the stroke-prone spontaneously hypertensive rats (SHRSP) and normal spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) of a Japanese colony. Two F_2 cohorts were independently produced: F_2(SHRSP × WKY) (110 male and 110 female rats) and F_2(SHR × WKY) (151 male rats). The ratio of heart weight to body weight (Hw/Bw) was evaluated at 12 months of age in F_2(SHRSP × WKY) after salt-loading for 7 months, and at around 15 weeks of age in F_2(SHR × WKY) who had been fed a normal rat chow diet. Subsequent to an initial screen with 251 markers in F_2(SHRSP × WKY) male progeny, 170 and 161 markers were selected and characterized in F_2(SHRSP × WKY) female progeny and F_2(SHR × WKY) male progeny, respectively. Markers from four chromosomal regions showed suggestive or significant linkage to Hw/Bw. The strongest and the most consistent linkage was found in the vicinity of D3Mgh16 on rat chromosome (RNO) 3 (a maximal log of the odds score reached 4.0 to 6.6 across the F_2 populations studied). In the other three regions on RNO6, RNO10 and RNO13, the degree of linkage was more prominent in either males or females. These data provide solid evidence for a "principal" RNO3 quantitative trait loci regulating Hw/Bw in SHRSP and SHR, and also suggest the possible presence of sexual dimorphism in regard to genetic susceptibility for cardiac hypertrophy. (*Hypertens Res* 2005; 28: 273–281)

***Key Words*: genetics, inbred strains, cardiac mass, hypertrophy, heart**

Introduction

Left ventricular hypertrophy is one of the major risk factors for cardiovascular morbidity and mortality, and considered to be the most important predictor of chronic heart failure (*1*). Several studies have investigated the influence of genetic background on the variability in left ventricular mass in humans, and the heritability of this trait has been estimated to be between 30% and 70% in different populations (*2*, *3*).

From the *[1]Department of Gene Diagnostics and Therapeutics, Research Institute, International Medical Center of Japan, Tokyo, Japan; *[2]Department of Cardiovascular Medicine, Tokyo Medical and Dental University, Tokyo, Japan; *[3]Graduate School of Medicine, the University of Tokyo, Tokyo, Japan; *[4]Graduate School of Medicine, Kyoto University, Kyoto, Japan; *[5]Department of Functional Pathology, School of Medicine, Shimane University, Izumo, Japan; *[6]School of Human Environmental Sciences, Mukogawa Women's University, Nishinomiya, Japan; and *[7]WHO Collaborating Center for Research on Primary Prevention of Cardiovascular Diseases, Kyoto, Japan.

This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 13204095) and a grant for Core Research for Evolutional Science and Technology from the Japan Science Technology Agency.

Address for Reprints: Norihiro Kato, M.D., Ph.D., Department of Gene Diagnostics and Therapeutics, Research Institute, International Medical Center of Japan, 1–21–1 Toyama, Shinjuku-ku, Tokyo 162–8655, Japan. E-mail: nokato@ri.imcj.go.jp

Received December 15, 2004; Accepted in revised form January 17, 2005.

Table 1. Phenotypic Traits in Three Parental Strains

	Male				
	SHRSP	SHR	WKY	*t*-test *p*-value	
				SHRSP *vs.* WKY	SHR *vs.* WKY
Hw (g)	0.980±0.083	0.880±0.022	0.784±0.062	0.0047	0.0232
Bw (g)	245.2±13.0	254.0±12.3	318.0±13.3	<0.0001	<0.0001
Hw/Bw (%)	0.41±0.02	0.35±0.01	0.25±0.02	<0.0001	<0.0001
SBP(mmHg)	219.5±15.3	181.8±3.5	131.7±4.5	<0.0001	<0.0001

	Female				
	SHRSP	SHR	WKY	*t*-test *p*-value	
				SHRSP *vs.* WKY	SHR *vs.* WKY
Hw (g)	0.777±0.103	0.675±0.094	0.554±0.040	0.0019	0.0289
Bw (g)	160.4±8.1	173.5±4.6	200.8±6.9	<0.0001	<0.0001
Hw/Bw (%)	0.48±0.1	0.39±0.1	0.28±0.02	<0.0001	0.004
SBP(mmHg)	200.1±19.6	152.2±12.0	127.0±11.5	<0.0001	0.009

Values are mean±SD. All phenotypic traits were measured for 4 or 5 rats in each strain at 13 weeks of age. SHR, spontaneously hypertensive rat; SHRSP, stroke-prone SHR; WKY, Wistar-Kyoto rat; Hw, heart weight; Bw, body weight; SBP, systolic blood pressure.

Body weight has been demonstrated to be positively correlated with left ventricular mass in adults of all ages, and this trait is also highly heritable (*4*).

However, because of the confounding influences of blood pressure and environmental factors such as dietary habits, it is not feasible to unravel the genetic determinants of cardiac mass in humans. The distribution of cardiac mass is mostly continuous within populations (*5*), suggesting that the trait is polygenic in nature, presumably involving multiple genes with each exerting independent and modest effects. This may hamper the detection of susceptibility genes by linkage analysis in humans. As an experimental alternative, genetic investigation of inbred strains of hypertensive rats has proven a powerful tool to identify loci that are involved in blood pressure regulation and cardiovascular disease phenotypes. Tanase *et al*. (*6*) previously conducted a detailed segregation study of 23 inbred normotensive and hypertensive rat strains and estimated that 65% to 75% of the strain difference in heart weight' was genetically determined. Several studies have therefore performed a segregation and genetic linkage analysis using F_2 progeny derived from a pair of progenitor strains that exhibit a substantial difference in adult cardiac mass (*7*). In most cases, these studies have analyzed cosegregation in crosses between a hypertensive and a normotensive strain, and have reported quantitative trait loci (QTLs) for cardiac mass or cardiac hypertrophy in a number of chromosomal regions (*8–10*).

Since the presence of sexual dimorphism has been implied for QTLs controlling blood pressure and related traits (*11–13*), we have undertaken a genome-wide linkage investigation of the genetic relationships between cardiac mass and body weight in male and female F_2 populations bred from the stroke-prone spontaneously hypertensive rat (SHRSP), one of the principal experimental models of hereditary hypertension and cardiovascular disease phenotypes, including cardiac hypertrophy. To examine the reproducibility of linkage between different inbred strains of particular hypertensive rats, we have additionally performed a genome-wide screen in male F_2 populations derived from the spontaneously hypertensive rat (SHR) of a Japanese colony. The Wistar Kyoto rat (WKY) has been used as a control progenitor strain to produce F_2 cohorts in all instances.

Methods

Animal Procedures

The SHRSP, SHR and WKY rat colonies have been maintained at our institute with brother-sister mating. By crossbreeding, two F_2 cohorts were independently produced from pairs of progenitor rats and used in genome-wide screens. The first cohort—F_2(SHRSP×WKY)—comprised 110 male and 110 female rats, and the second cohort—F_2(SHR×WKY)—comprised 151 male rats. Rats were weaned at 4 weeks after birth and were placed on an SP diet (Funabashi Farms, Funabashi, Japan) containing 5% fat, 0.4% sodium, and 0.75% potassium. In F_2(SHRSP×WKY), salt-loading was conducted after 5 months of age with the rats being given 1% NaCl in drinking water for 7 months, and the rats were killed by exsanguination under pentobarbital anesthesia at 12 months of age. Systolic blood pressure (SBP) was measured by the tail cuff method at 13 weeks of age as previously described (*11*). On the day of tissue collection, the total body weight and heart weight of each rat were measured and recorded in g, and pieces of the liver were frozen at −70°C for subsequent DNA extraction. In F_2(SHR×WKY), on the

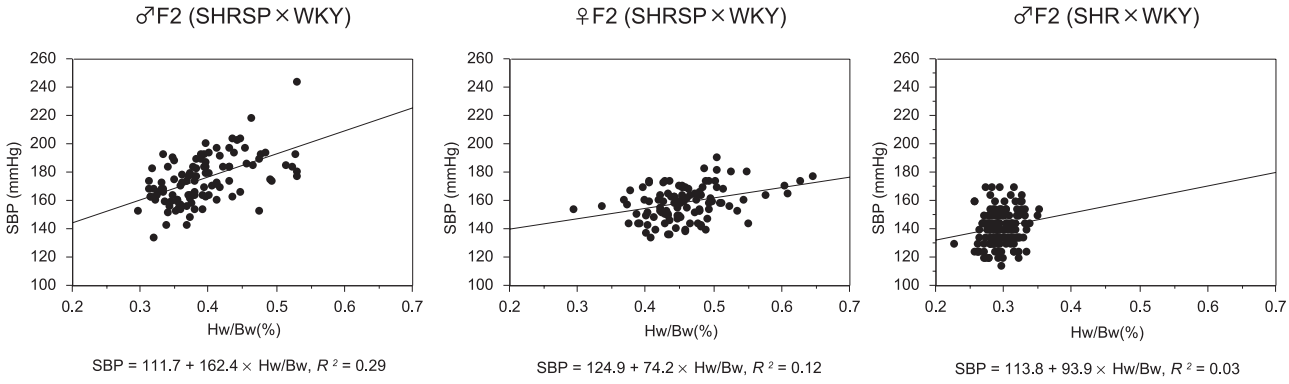

Fig. 1. *Scatter plots showing correlations between hemodynamic overload (represented by systolic blood pressure [SBP]) and the degree of cardiac hypertrophy (represented by Hw/Bw). SBP was measured at 13 weeks of age in F_2(SHRSP×WKY) and at 11 weeks of age in F_2(SHR×WKY), whereas Hw/Bw was evaluated at 12 months of age in F_2(SHRSP×WKY) and around 15 weeks of age in F_2(SHR×WKY) (see Methods).*

other hand, SBP was measured at 11 weeks of age and the rats were killed under pentobarbital anesthesia at around 15 weeks of age. Although the timing of weight measurement was slightly varied in F_2(SHR×WKY) because of an *in vitro* assay using the adipocytes isolated from individual rats (which is part of our ongoing project concerning insulin resistance phenotypes), our preliminary data showed that this variation did not exert a considerable influence on total body weight or heart weight in the progenitor strains. All rats were laboratory animals and were treated in compliance with local regulations. This protocol was approved by the animal ethics committee of the Research Institute of the International Medical Center of Japan.

Genotype Characterization

Genotyping was carried out using microsatellite markers amplified by polymerase chain reaction (PCR) and evaluated by electrophoresis as previously described (*11*, *14*). Markers were chosen for a genome screen on the basis of their location in the published genetic maps (*15*, *16*) to avoid genotyping closely linked markers of the same chromosomal region. Genome-wide searches were carried out in two F_2 cohorts. At first, a total of 251 microsatellite markers distributed across 20 rat autosomes and the rat chromosome X were characterized in the male progeny of F_2(SHRSP×WKY). The typed markers spanned 1,790 centimorgan (cM) of the rat genome, and the average spacing of the genome covered within 10 cM of a marker. Subsequently, based on the linkage map thus constructed in the male progeny of F_2(SHRSP×WKY) and by referring to the published genetic maps, 170 and 161 markers were selected for genome screens in the female progeny of F_2(SHRSP×WKY) and in the male progeny of F_2(SHR×WKY), respectively. In this selection, markers were spaced as evenly as possible to provide a marker every 10–20 cM.

Development of Polymorphic Markers on Rat Chromosome 3

After the initial genome screen, we attempted to develop new microsatellite markers to fill in the remaining gaps and to integrate our consensus linkage map with physical maps and ideograms of rat chromosome (RNO) 3. First, repetitive motifs (mostly di-nucleotide repeat sequences) were sought in the intron or the 5′- and 3′-untranslated regions of the annotated genes in the rat database (http://www.ncbi.nlm. nih.gov/genome/guide/rat/), which were primarily chosen based on their positions in the assembly map of RNO3. Then, PCR primer sets were designed to flank the target repeats and subjected to the examination of polymorphism among 3 progenitor strains. A genetic linkage map of RNO3 involving all markers that were polymorphic between SHRSP and WKY was constructed by genotyping 63 male F_1 backcross rats—F_1(SHRSP×WKY)×SHRSP—as previously reported (*17*).

Statistical Analysis

Genetic effects of each marker locus on heart weight, body weight and relative heart weight—heart weight divided by body weight (Hw/Bw)—were evaluated with one-way ANOVA, where Hw/Bw was considered as a quantitative trait representing cardiac hypertrophy. X-linked markers were analyzed apart from the autosomal markers according to the difference in parental origin of the marker alleles. Linkage maps were constructed by using the MAPMAKER/EXP 3.0 program (*18*) with an error detection procedure, and genetic distances were calculated with the Haldane's mapping function. Multipoint linkage analysis was performed by using the MAPMAKER/QTL 1.1 program (*18*). Percentages of the trait variance attributed to an individual marker (R^2) were calculated by linear regression analysis. The fraction of overall

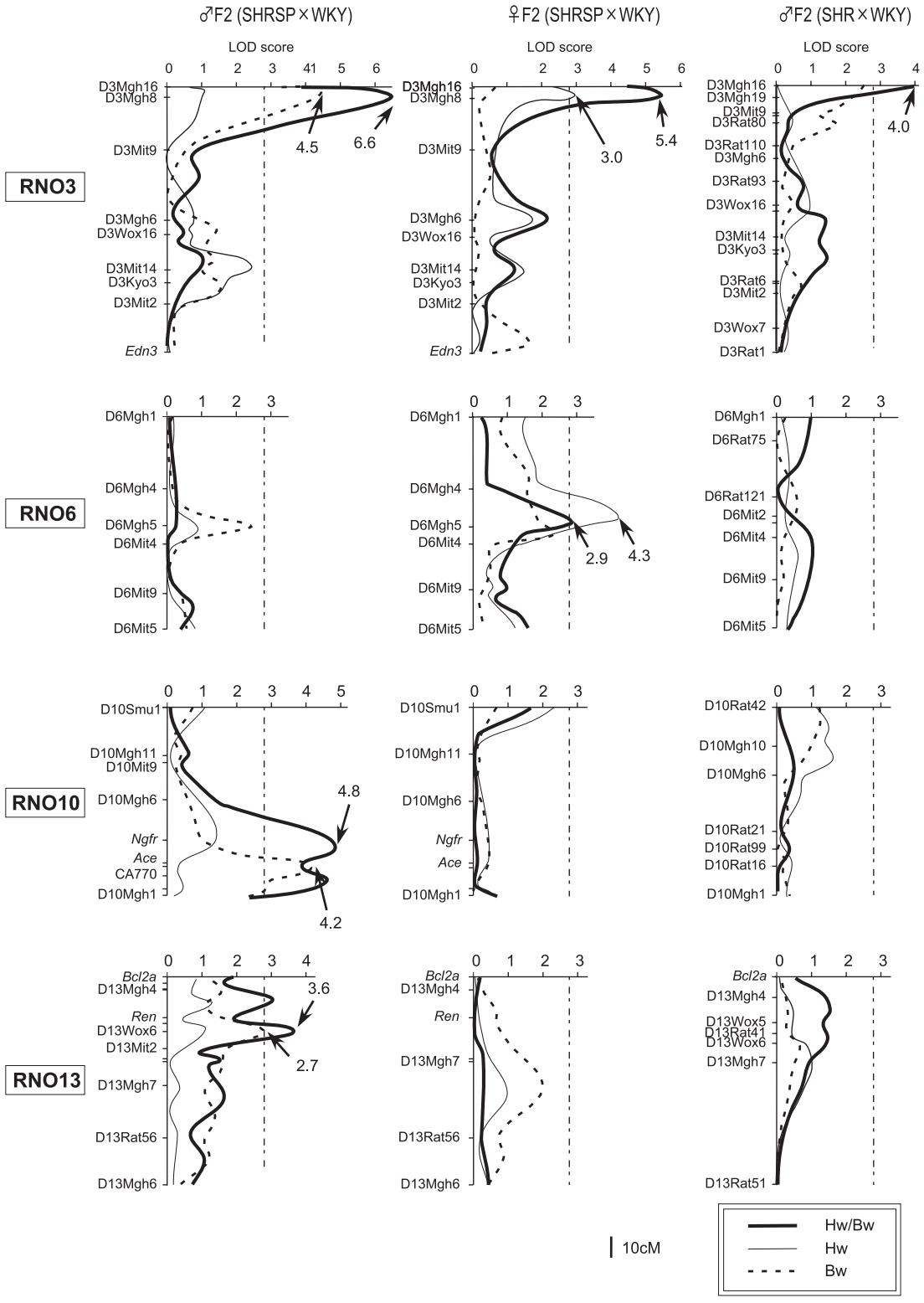

Fig. 2. *QTL plots of 4 chromosomes in male progeny (left) and female progeny (middle) of F_2(SHRSP×WKY) and male progeny of F_2(SHR×WKY) (right). Heart weight (Hw) and body weight (Bw) evaluated at 12 months of age in F_2(SHRSP×WKY) and around 15 weeks of age in F_2(SHR×WKY) were used for linkage analysis. LOD score plots are displayed for each of Hw/Bw (thick line), Hw (thin line), and Bw (thin dotted line). Distances between markers are map units in cM. For each chromosome, not all typed markers are shown for reason of readability. Vertical lines show a LOD score of 2.8 and the figures indicated are LOD scores for the highest QTL plot peak.*

Table 2. Mean Trait Values According to Genotypes in F_2 Populations Derived from SHRSP, SHR and WKY

Chromosome	Marker	Trait	F_2(SHRSP×WKY) Male (n=110)					F_2(SHRSP×WKY) Female (n=110)				
			SP/SP	SP/WKY	WKY/WKY	ANOVA p-value	Maximal LOD score	SP/SP	SP/WKY	WKY/WKY	ANOVA p-value	Maximal LOD score
3	D3Mgh8	Hw/Bw	0.44 (0.06)	0.37 (0.04)	0.36 (0.04)	2.93×10^{-8}	6.6	0.50 (0.07)	0.45 (0.04)	0.44 (0.04)	5.50×10^{-6}	5.4
	D3Mgh16	Hw/Bw	—	—	—	—	—	—	—	—	—	—
6	D6Mgh5	Hw/Bw	0.39 (0.07)	0.39 (0.05)	0.38 (0.05)	0.64	0.4	0.49 (0.07)	0.45 (0.05)	0.44 (0.05)	0.001	2.9
	D6Mit2	Hw/Bw	—	—	—	—	—	—	—	—	—	—
10	Ngfr	Hw/Bw	0.43 (0.06)	0.39 (0.05)	0.36 (0.04)	3.68×10^{-5}	4.8	0.46 (0.06)	0.46 (0.06)	0.45 (0.04)	0.87	0.1
	D10Rat21	Hw/Bw	—	—	—	—	—	—	—	—	—	—
13	D13Wox6	Hw/Bw	0.43 (0.06)	0.38 (0.05)	0.38 (0.05)	0.002	3.6	0.46 (0.05)	0.46 (0.06)	0.46 (0.07)	0.99	0
1	Arix	Bw	392 (51)	438 (45)	428 (45)	0.004	3.2	259 (29)	264 (19)	266 (21)	0.53	0.2
	D1Wox10	Bw	414 (53)	429 (49)	438 (43)	0.16	0.87	264 (27)	265 (20)	273 (33)	0.6	0.33
	D1Rat112	Bw	—	—	—	—	—	—	—	—	—	—
3	D3Mgh16	Bw	399 (46)	429 (42)	457 (47)	1.71×10^{-5}	4.5	259 (27)	266 (20)	264 (19)	0.38	0.4
10	CA770	Bw	400 (44)	428 (43)	447 (43)	0.0008	4.2	268 (26)	265 (21)	259 (21)	0.36	0.5
	D10Rat13	Bw	—	—	—	—	—	—	—	—	—	—
18	D20Mgh2L	Bw	443 (51)	434 (41)	397 (49)	0.0005	3.4	264 (12)	268 (20)	256 (31)	0.12	1.2
	D18Mgh4	Bw	—	—	—	—	—	—	—	—	—	—

Chromosome	Marker	Trait	F_2(SHR×WKY) Male (n=151)				
			SR/SR	SR/WKY	WKY/WKY	ANOVA p-value	Maximal LOD score
3	D3Mgh8	Hw/Bw	—	—	—	—	—
	D3Mgh16	Hw/Bw	0.31 (0.02)	0.29 (0.02)	0.29 (0.02)	0.0001	4.0
6	D6Mgh5	Hw/Bw	—	—	—	—	—
	D6Mit2	Hw/Bw	0.29 (0.02)	0.29 (0.02)	0.30 (0.02)	0.57	0.26
10	Ngfr	Hw/Bw	—	—	—	—	—
	D10Rat21	Hw/Bw	0.29 (0.02)	0.30 (0.02)	0.29 (0.03)	0.88	0.1
13	D13Wox6	Hw/Bw	0.30 (0.02)	0.29 (0.02)	0.29 (0.02)	0.04	1.5
1	Arix	Bw	291(21)	288(25)	296(22)	0.27	0.59
	D1Wox10	Bw	—	—	—	—	—
	D1Rat112	Bw	278 (19)	293 (22)	298 (26)	0.0003	3.5
3	D3Mgh16	Bw	280 (21)	295 (23)	294 (24)	0.0036	2.6
10	CA770	Bw	—	—	—	—	—
	D10Rat13	Bw	287 (25)	293 (25)	290 (22)	0.47	0.3
18	D20Mgh2L	Bw	—	—	—	—	—
	D18Mgh4	Bw	295 (22)	291 (25)	289 (23)	0.5	0.3

Values are trait mean with SD being described in the parentheses. Hw/Bw (relative heart weight) and Bw (body weight) are shown as percentage (%) and gram (g), respectively. Markers with the most significant ANOVA p-values are selected from chromosomal regions showing linkage (maximal LOD score ≥2.8) to Hw/Bw and Bw in any of the 3 F_2 populations. When a marker is informative in F_2(SHRSP×WKY) but not in F_2(SHR×WKY), its closest marker is alternatively shown in F_2(SHR×WKY) and the data cells are denoted as blank "—" in the corresponding marker. D20Mgh2L is mapped to a region on rat chromosome 18 in accordance with the previous report by Bihoreau *et al.* (*15*). SP, SHRSP; SR, SHR; other abbreviations are the same as in Table 1.

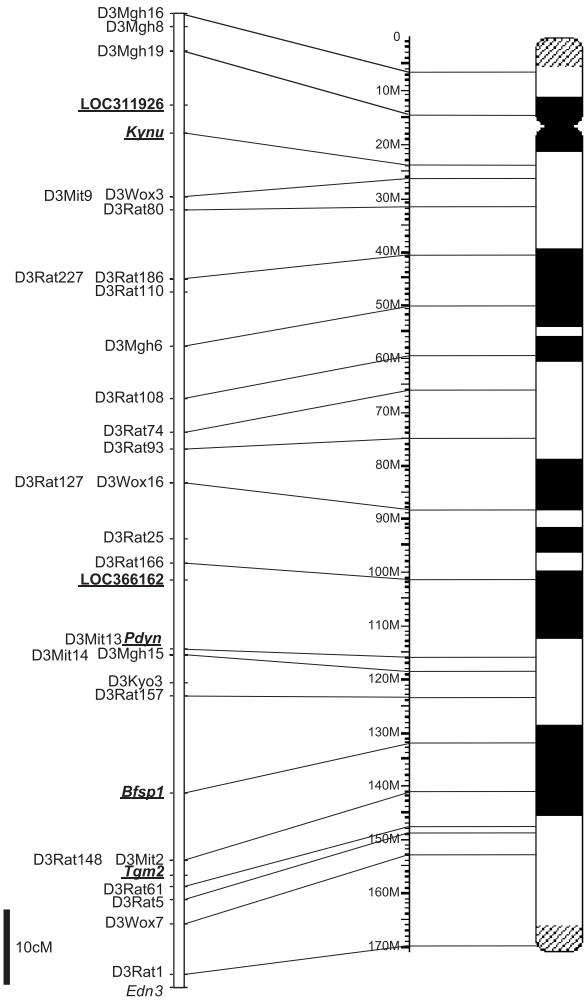

Fig. 3. *Comparison of a linkage map with physical maps and ideograms of RNO3. The six microsatellite markers newly developed in the present study are underlined to the left of the linkage map.*

variance collectively attributable to identified QTLs was calculated by multiple regression analysis. The statistical threshold recommended by Lander and Kruglyak (*19*) was used to declare linkage; *i.e.*, a nominal p-value of 1.6×10^{-3} or a log of the odds (LOD) score of 2.8 for "suggestive" linkage, and a nominal p-value of 5.2×10^{-5} or a LOD score of 4.3 for "significant" linkage.

Results

The baseline characteristics of 3 progenitor strains—SHRSP, SHR and WKY—are shown in Table 1. SBP and heart weight were significantly higher and body weight was significantly lower in both SHRSP and SHR than in WKY. Accordingly, the strain differences were more prominent in Hw/Bw than in heart weight. The relationship between hemodynamic overload and the degree of cardiac hypertrophy is depicted in the scatter plots (Fig. 1). Here, because there were relatively fair correlations between any two points of blood pressure measurements during the longitudinal study in F_2(SHRSP×WKY) (correlation coefficients 0.57–0.70, $p<0.0001$), a single point of blood pressure measurements was chosen to represent hemodynamic overload. The rats were exposed to considerably high blood pressure (we used representative values measured at 13 weeks of age) for a longer time and also exposed to salt-loading in F_2(SHRSP×WKY), whereas the rats were exposed to modestly high blood pressure (we used representative values measured at 11 weeks of age) for a shorter time on a normal rat chow diet in F_2(SHR×WKY) (see Methods). As a consequence, a wider distribution of Hw/Bw and its closer correlation with representative blood pressure values were observed in F_2(SHRSP×WKY) than in F_2(SHR×WKY).

A total of four regions showing suggestive or significant linkage to Hw/Bw were identified on four different chromosomes (Fig. 2 and Table 2). In any of the F_2 populations, the strongest evidence of linkage was consistently found in the region on RNO3. Male-specific linkage was detected in the region on RNO10 (around *Ace*) in the F_2(SHRSP×WKY) cohort alone. Also, markers from the regions on RNO6 and RNO13 showed suggestive evidence of linkage in a sex-specific and cohort-specific manner. To investigate the genetic impacts of individual QTLs, percentages of the Hw/Bw variance attributed to each possible QTL (R^2) were calculated in the F_2 populations. The RNO3 QTL accounted for 23% and 17% of the Hw/Bw variance in male and female progeny of F_2(SHRSP×WKY) and 8% of the variance in male progeny of F_2(SHR×WKY), respectively. The highest fraction of overall variance collectively attributable to a set of linked regions was 47% in male progeny of F_2(SHRSP×WKY). There was no significant epistatic interaction between the Hw/Bw QTLs in either sex (data not shown).

In addition to the ratio, *i.e.*, Hw/Bw, the numerator (Hw) and denominator (Bw) were separately examined as quantitative traits in genome screens. Significant linkage was found for heart weight in the female progeny of F_2(SHRSP×WKY) on RNO6 and for body weight in the male progeny of F_2(SHRSP×WKY) on RNO3. Markers from these regions were cosegregated with Hw/Bw in the corresponding F_2 progeny as mentioned above. Among the other chromosomal regions showing suggestive or significant evidence of linkage to Hw/Bw, markers were cosegregated with heart weight in the female progeny of F_2(SHRSP×WKY) on RNO3 and with body weight in the male progeny of F_2(SHRSP×WKY) on RNO10 and RNO13.

Because genome screens consistently provided significant evidence in favor of the RNO3 QTL for Hw/Bw, we next constructed a consensus linkage map of RNO3 involving markers polymorphic between SHRSP and WKY. A total of six microsatellite markers were newly developed and these reduced the gaps that had remained in the initial genome screens to less than 15 cM across the chromosome (Fig. 3 and Table 3).

Table 3. Primers Designed in This Study

Marker	Primer sequences (5′- to -3′)	Length (mer)	PCR conditions (anneal temp./$MgCl_2$)	PCR product size (bp)		
				SHRSP	SHR	WKY
Kynu [1]	TATGCTCTTTTTTCTCACAGAAAC	24	TD 66–58°C/2 mmol/l	158	152	144
	TCCACCCTCATAATATGTTCTC	22				
Kynu [2]	CCGCTAGAATGAATTGATATAC	22	TD 66–58°C/2 mmol/l	189	189	191
	CTCATGGCCATAGGCTC	17				
Tgm2	TGATAATGGTGCATTGTACCTGAA	24	TD 66–58°C/2 mmol/l	131	135	127
	GGCCAAATGTTCTTTCTTAGTAT	23				
LOC311926	AAGAGTCATCTAGTTACGAGAAA	23	TD 66–58°C/2 mmol/l	257	257	255
	ATCTAGGAATTTTTGATTAGTAATA	25				
Pdyn	CAATTTGTTTGGAGAAAAGTCA	22	TD 66–58°C/2 mmol/l	190	190	188
	TTTTTCCAAATTGTTTGAGC	20				
Bfsp1	AAGACTATAGAGAGGCCATTAG	22	TD 66–58°C/2 mmol/l	225	225	227
	GTCAAGGGCTTCATCTAGG	19				
LOC366162	CCTCACTAAGCTACTTGCTA	20	60°C/2 mmol/l	188	188	192
	GAGATGATGCTACTGATGTATT	22				

TD represents a touch-down PCR method. Two primer sets were designed for different CA-repeats in the *Kynu* locus. *Kynu*, kynureninase; *Tgm2*, tissue-type transglutaminase; *Pdyn*, prodynorphin; *Bfsp1*, beaded filament structural protein 1; temp., temperature; PCR, polymerase chain reaction; SHR, spontaneously hypertensive rat; SHRSP, stroke-prone SHR; WKY, Wistar-Kyoto rat.

Discussion

The present study provides several notable insights into the genetics of cardiac hypertrophy using animal models. First, the genome-wide searches provided solid evidence of linkage to the relatively narrow region (<25 cM) on RNO3 in two F_2 cohorts independently produced from SHRSP and SHR. Second, the relevant linkage was replicated despite differences in confounding factors, such as hemodynamic overload (the duration of exposure and the degree of blood pressure elevation) and dietary manipulation (with or without salt-loading). Third, it is of interest that three out of four QTLs were detected in either of the sexes, indicating the possible presence of sexual dimorphism.

Reproducibility of linkage has been debated in molecular genetics of cardiovascular disease traits such as hypertension (*7*). Apart from the present study, several genome-wide screens have been carried out in F_2 populations derived from inbred rat strains and have reported QTLs contributing to the regulation of adult cardiac mass independent of blood pressure on RNO1 (*20*), RNO2 (*9*), RNO3 (*20*, *21*), RNO5 (*22*), RNO7 (*23*), RNO8 (*24*), RNO9 (*20*), RNO10 (*25*, *26*), RNO12 (*27*, *28*), RNO14 (*12*), RNO17 (*10*, *23*), RNO18 (*29*) and RNO19 (*20*). As for the results obtained from SHR-derived crosses, there exists no apparent overlapping of linkage among the studies reported to date. In this context, the present study has, for the first time, identified reproducible linkage to cardiac mass on RNO3 in two F_2 cohorts independently produced from SHR substrains—SHRSP and normal SHR—of a Japanese colony. Of particular note is the fact that this RNO3 linkage is replicated not only between male and female progeny of F_2(SHRSP×WKY) but also between F_2(SHRSP×WKY) and F_2(SHR×WKY) male progeny. With regard to SBP linkage in the relevant regions on RNO3, no significant QTL plot peaks have been detected either in F_2(SHRSP×WKY) as previously reported (*11*) or in F_2(SHR×WKY) (data not shown). Also, it should be noted that, while significant linkage to body weight (*i.e.*, a denominator of Hw/Bw) and suggestive linkage to heart weight (*i.e.*, a numerator of Hw/Bw) are solely detectable on RNO3 in male and female progeny of F_2(SHRSP×WKY), respectively, substantial evidence of linkage to Hw/Bw is observed across the F_2 populations studied. This may indicate that the ratio, *i.e.*, Hw/Bw, is not a derivative trait but can be regarded as a unique quantitative trait.

In the design of linkage analysis, we have focused on three confounding factors—sex, the extent of hemodynamic load, and salt-loading—as previously discussed in human studies (*30*–*33*). It may be argued that these confounding factors complicate the interpretation of strain differences between SHRSP and SHR, as discussed above. Nevertheless, the reproducible results of linkage seem to strengthen the possibility that the RNO3 QTL could constitute a "principal" genetic determinant of relative cardiac mass in SHR as a whole. The previous observations by Siegel *et al*. (*20*) further support this possibility. That is, they reported a significant QTL for left ventricular weight (a maximal LOD score of 7.34) in the vicinity of D3Mgh9 (which is close to D3Mgh8 in the present study) by performing genome screens in the F_2 intercross between the Dahl salt sensitive rat and SHR under a salt-loading condition. With reference to our consensus linkage map (Fig. 3), we have searched the rat genome databases to identify potential candidate genes on RNO3 and have

noticed a few candidate genes, *i.e.*, *Dbh* (dopamine β hydroxylase), *Kynu* (kynureninase) and *Angptl2* (angiopoietin-like 2).

Our results highlight the importance of sexual dimorphism (*i.e.*, some QTLs for Hw/Bw were found in females alone and others in males alone). Only a few genome-wide linkage studies have so far attempted to analyze the results separately by sex. Accordingly, even if sex-specific QTLs actually existed, they may have been missed when either of the sexes was used for a linkage study. As we have previously reported significant linkage to SBP on RNO10 (near *Ace*) and RNO13 (near *Ren*) in the male progeny of F_2(SHRSP×WKY) (*11*, *34*), we cannot draw any definite conclusions as to whether these loci confer genetic susceptibility to cardiac hypertrophy independently of systemic arterial pressure in the current linkage analysis by itself.

In conclusion, we report the results of genome-wide screens to explore chromosomal regions that control the difference in cardiac mass between SHR-substrains and normotensive WKY. We have identified a principal QTL on RNO3 as well as three possible QTLs on RNO6, RNO10 and RNO13. Detailed investigation of these chromosomal regions through the construction of congenic rats would help to clarify the genetic mechanisms underlying the development of cardiac hypertrophy and would thus seem to be of clinical importance (*35*).

References

1. Arnett DK, de las Fuentes L, Broeckel U: Genes for left ventricular hypertrophy. *Curr Hypertens Rep* 2004; **6**: 36–41.
2. Verhaaren HA, Schieken RM, Mosteller M, Hewitt JK, Eaves LJ, Nance WE: Bivariate genetic analysis of left ventricular mass and weight in pubertal twins (the Medical College of Virginia twin study). *Am J Cardiol* 1991; **68**: 661–668.
3. Post WS, Larson MG, Myers RH, Galderisi M, Levy D: Heritability of left ventricular mass: the Framingham Heart Study. *Hypertension* 1997; **30**: 1025–1028.
4. Gardin JM, Henry WL, Savage DD, Ware JH, Burn C, Borer JS: Echocardiographic measurements in normal subjects: evaluation of an adult population without clinically apparent heart disease. *J Clin Ultrasound* 1979; **7**: 439–447.
5. Chaturvedi N, Athanassopoulos G, McKeigue PM, Marmot MG, Nihoyannopoulos P: Echocardiographic measures of left ventricular structure and their relation with rest and ambulatory blood pressure in blacks and whites in the United Kingdom. *J Am Coll Cardiol* 1994; **24**: 1499–1505.
6. Tanase H, Yamori Y, Hansen CT, Lovenberg W: Heart size in inbred strains of rats. Part 1. Genetic determination of the development of cardiovascular enlargement in rats. *Hypertension* 1982; **4**: 864–872.
7. Rapp JP: Genetic analysis of inherited hypertension in the rat. *Physiol Rev* 2000; **80**: 135–172.
8. Kato N, Hyne G, Bihoreau MT, Gauguier D, Lathrop GM, Rapp JP: Complete genome searches for quantitative trait loci controlling blood pressure and related traits in four segregating populations derived from Dahl hypertensive rats. *Mamm Genome* 1999; **10**: 259–265.
9. Innes BA, McLaughlin MG, Kapuscinski MK, Jacob HJ, Harrap SB: Independent genetic susceptibility to cardiac hypertrophy in inherited hypertension. *Hypertension* 1998; **31**: 741–746.
10. Pravenec M, Gauguier D, Schott JJ, *et al*: Mapping of quantitative trait loci for blood pressure and cardiac mass in the rat by genome scanning of recombinant inbred strains. *J Clin Invest* 1995; **96**: 1973–1978.
11. Kato N, Mashimo T, Nabika T, Cui ZH, Ikeda K, Yamori Y: Genome-wide searches for blood pressure quantitative trait loci in the stroke-prone spontaneously hypertensive rat of a Japanese colony. *J Hypertens* 2003; **21**: 295–303.
12. Clark JS, Jeffs B, Davidson AO, *et al*: Quantitative trait loci in genetically hypertensive rats. Possible sex specificity. *Hypertension* 1996; **28**: 898–906.
13. Yagil C, Sapojnikov M, Kreutz R, *et al*: Salt susceptibility maps to chromosomes 1 and 17 with sex specificity in the Sabra rat model of hypertension. *Hypertension* 1998; **31**: 119–124.
14. Kato N, Tamada T, Nabika T, *et al*: Identification of quantitative trait loci for serum cholesterol levels in stroke-prone spontaneously hypertensive rats. *Arterioscler Thromb Vasc Biol* 2000; **20**: 223–229.
15. Bihoreau MT, Gauguier D, Kato N, *et al*: A linkage map of the rat genome derived from three F2 crosses. *Genome Res* 1997; **7**: 434–440.
16. Jacob HJ, Brown DM, Bunker RK, *et al*: A genetic linkage map of the laboratory rat, *Rattus norvegicus*. *Nat Genet* 1995; **9**: 63–69.
17. Kato N, Nabika T, Liang YQ, *et al*: Isolation of a chromosome 1 region affecting blood pressure and vascular disease traits in the stroke-prone rat model. *Hypertension* 2003; **42**: 1191–1197.
18. Lander ES, Green P, Abrahamson J, *et al*: MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1987; **1**: 174–181.
19. Lander E, Kruglyak L: Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995; **11**: 241–247.
20. Siegel AK, Planert M, Rademacher S, *et al*: Genetic loci contribute to the progression of vascular and cardiac hypertrophy in salt-sensitive spontaneous hypertension. *Arterioscler Thromb Vasc Biol* 2003; **23**: 1211–1217.
21. Sebkhi A, Zhao L, Lu L, Haley CS, Nunez DJ, Wilkins MR: Genetic determination of cardiac mass in normotensive rats: results from an F344×WKY cross. *Hypertension* 1999; **33**: 949–953.
22. Deschepper CF, Masciotra S, Zahabi A, Boutin-Ganache I, Picard S, Reudelhuber TL: Functional alterations of the Nppa promoter are linked to cardiac ventricular hypertrophy in WKY/WKHA rat crosses. *Circ Res* 2001; **88**: 223–228.
23. Tsujita Y, Iwai N, Tamaki S, Nakamura Y, Nishimura M, Kinoshita M: Genetic mapping of quantitative trait loci influencing left ventricular mass in rats. *Am J Physiol Heart Circ Physiol* 2000; **279**: H2062–H2067.
24. Kren V, Pravenec M, Lu S, *et al*: Genetic isolation of a region of chromosome 8 that exerts major effects on blood pressure and cardiac mass in the spontaneously hypertensive

rat. *J Clin Invest* 1997; **99**: 577–581.
25. Hamet P, Kaiser MA, Sun Y, *et al*: HSP27 locus cosegregates with left ventricular mass independently of blood pressure. *Hypertension* 1996; **28**: 1112–1117.
26. Zhang L, Summers KM, West MJ: Analysis of linkage of the ACE locus with measures of cardiac hypertrophy in the spontaneously hypertensive rat. *Clin Exp Pharmacol Physiol* 1996; **23**: 597–599.
27. Zhang L, Summers KM, West MJ: Angiotensin I converting enzyme gene cosegregates with blood pressure and heart weight in F2 progeny derived from spontaneously hypertensive and normotensive Wistar-Kyoto rats. *Clin Exp Hypertens* 1996; **18**: 753–771.
28. Harris EL, Phelan EL, Thompson CM, Millar JA, Grigor MR: Heart mass and blood pressure have separate genetic determinants in the New Zealand genetically hypertensive (GH) rat. *J Hypertens* 1995; **13**: 397–404.
29. Katsuya T, Takami S, Higaki J, *et al*: Gap junction protein locus on chromosome 18 cosegregates with body weight in the spontaneously hypertensive rat. *Hypertens Res* 1995; **18**: 63–67.
30. Kato N, Kanda T, Sagara M, *et al*: Proposition of a feasible protocol to evaluate salt sensitivity in a population-based setting. *Hypertens Res* 2002; **25**: 801–809.
31. O'Donnell CJ, Lindpaintner K, Larson MG, *et al*: Evidence for association and genetic linkage of the angiotensin-converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham Heart Study. *Circulation* 1998; **97**: 1766–1772.
32. Higaki J, Baba S, Katsuya T, *et al*: Deletion allele of angiotensin-converting enzyme gene increases risk of essential hypertension in Japanese men: the Suita Study. *Circulation* 2000; **101**: 2060–2065.
33. Katsuya T, Ishikawa K, Sugimoto K, Rakugi H, Ogihara T: Salt sensitivity of Japanese from the viewpoint of gene polymorphism. *Hypertens Res* 2003; **26**: 521–525.
34. Mashimo T, Nabika T, Matsumoto C, *et al*: Aging and salt-loading modulate blood pressure QTLs in rats. *Am J Hypertens* 1999; **12**: 1098–1104.
35. Morgan T: Renin, angiotensin, sodium and organ damage. *Hypertens Res* 2003; **26**: 349–354.