Genetically Hypertensive Brown Norway Congenic Rat Strains Suggest Intermediate Traits Underlying Genetic Hypertension

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Aim To determine the independent and combined effects of three quantitative trait loci (QTL) for blood pressure in the Genetically Hypertensive (GH/Omr) rat by generating and characterizing single and combined congenic strains that have QTL on rat chromosomes (RNO) 2, 6, and 18 from the GH rat introduced into a hypertension resistant Brown Norway (BN) background.

Methods Linkage analysis and QTL identification (genome wide QTL scan) were performed with MapMaker/EXP to build the genetic maps and MapMaker/QTL for linking the phenotypes to the genetic map. The congenic strains were derived using marker-assisted selection strategy from a single male F1 offspring of an intercross between the male GH/Omr and female BN/Elh, followed by 10 generations of selective backcrossing to the female BN progenitor strain. Single congenic strains generated were BN.GH-(*D2Rat22-D2Mgh11*)/Mcwi (BN.GH2); BN.GH-(*D6Mit12-D6Rat15*)/Mcwi (BN.GH6); and BN.GH-(*D18Rat41-D18Mgh4*)/Mcwi (BN.GH18). Blood pressure measurements were obtained either via a catheter placed in the femoral artery or by radiotelemetry in the single and combined congenics. Responses to angiotensin II (ANGII), norepinephrine (NE), and baroreceptor sensitivity were measured in the single congenics.

Results Transferring one or more QTL from the hypertensive GH into normotensive BN strain was not sufficient to cause hypertension in any of the developed congenic strains. There were no differences between the parental and congenic strains in their response to NE. However, BN.GH18 rats revealed significantly lower baroreceptor sensitivity ($\beta = -1.25 \pm 0.17$), whereas BN.GH2 ($\beta = 0.66 \pm 0.09$) and BN.GH18 ($\beta = 0.71 \pm 0.07$) had significantly decreased responses to ANGII from those observed in the BN ($\beta = 0.88 \pm 0.08$).

Conclusion The failure to alter blood pressure levels by introducing the hypertensive QTL from the GH into the hypertension resistant BN background suggests that the QTL effects are genome background-dependent in the GH rat. BN.GH2 and BN.GH18 rats reveal significant differences in response to ANGII and impaired baroreflex sensitivity, suggesting that we may have captured a locus responsible for the genetic control of baroreceptor sensitivity, which would be considered an intermediate phenotype of blood pressure.

It is widely known that genetic factors play an important role in the onset and progression of hypertension and hypertension-induced end-organ damage in humans (1). As with all common multifactorial diseases, identifying the genetic components of hypertension in humans is complicated by allelic, locus, and epidemiological heterogeneity, as well as by a significant environmental component (2,3). Heritability measures for human essential hypertension range from 15%-60% (4,5). Main approaches used to dissect genetics of hypertension include linkage analysis in families with hypertension, linkage studies in affected sib-pairs, and candidate gene and genome-wide association studies in candidate genes (6,7). However, with the exception of rare monogenic forms of hypertension such as Liddle's syndrome (8), the syndrome of apparent mineralocorticoid excess (9), and glucocorticoid remediable aldosteronism (10), identification of the major genetic factors of essential hypertension remains elusive (11). In addition to genome-wide scans and association studies, the use of "intermediate" phenotypes is a relatively common tool proposed to help unravel the genetic basis of hypertension. Unfortunately, despite widespread discussion of using "intermediate" phenotypes (12,13), there is little hard evidence that they exist (14). Often what is thought to be an intermediate phenotype is in itself a complex trait (15,16).

Several inbred hypertensive rat strains have been independently developed to mimic various aspects of the pathogenesis of human hypertension (17), with the expectation that the reduced heterogeneity in the rat will facilitate quantitative trait loci (QTL) and gene identification, which can then be followed-up in humans. In this study, we investigated the Genetically Hypertensive rat (GH/Omr). The GH rat was the first genetically inbred rat model for hypertension, characterized by early onset of hypertension (by the age of 6 weeks), hypercholesterolemia, cardiac hypertrophy, and vascular disease (18).

Here we report the first complete genome linkage scan of hypertension and related traits in the GH rat and results of the initial physiological characterization of the single, double, and triple congenics, in comparison with the parental GH and BN strains.

Material and methods

Animals

All breeding for the linkage studies was done at the University of Otago Animal Breeding Station, as previously described by Harris et al (19), under approved animal protocols. Breeding for physiological characterization in single, double, and triple congenics was done at the Animal Research Center of the Medical College of Wisconsin (MCW), Milwaukee, WI, USA. Animals were housed in standard rat cages with lights in the Animal Care Facility on from 6:00 AM to 6:00 PM, which was approved by the American Association for the Accreditation of Laboratory Animal Care. All protocols were approved by the Medical College of Wisconsin's Animal Welfare Committee. Standard commercial rat chow (LabDiet chow – Purina Mills, Virginia, USA, catalog No. 5001, 0.4% NaCl) and drinking fluid (tap water) were provided *ad libitum*.

Construction of single congenic animals was initiated in New Zealand, derived from a single male F1 offspring of an intercross between the male GH/Omr and female BN/Elh (19). The male F1 offspring was then backcrossed to a female BN. Partially congenic rats were exported to Charles River Laboratories, Wilmington, MA, USA, for cesarean rederivation and then sent to MCW in 1999. The initial congenic strains were developed after 10 generations of selective backcrossing to the female BN progenitor strain, followed by a final intercross to fix the QTL segment of GH

genome. The size of introgressed segment was finally determined by genotyping using selected simple sequence length polymorphisms (SSLPs). We tested each strain with 112 genetic markers distributed across the genome to ensure the genome background was that of the BN rat. All congenics were maintained by brother/sister mating. The double and triple congenics were derived at MCW by intercrossing single congenics. We used a marker-assisted selection strategy to build the single and double congenic strains (20-22) and screened SSLPs within and by flanking the 99% confidence interval for each of the blood pressure QTL.

Genotyping

Genomic DNA was extracted from the tail tips of the backcross and intercross progeny by standard methods (23). The DNA was diluted to 5 ng/μL stocks in sterile, distilled water. Genotyping with SSLPs was performed using radioactive P^{32} labeled markers as previously described (24).

Genetic mapping

We performed a total genome scan using 260 SSLPs markers corresponding to approximately one marker every 5.6 centiMorgan (cM) by a two step linkage analysis strategy. Initially, we performed a genome scan using the 46 most phenotypically divergent and informative F2 animals to identify putative QTL. The putative QTL were then validated by genotyping the remaining 61 animals with all markers in the regions of interest and reanalyzing linkage with all animals. Prior to the linkage analysis, the distribution of the phenotypes in the F2 population was tested for normality using a Kolmogorov-Smirnov test (25). Traits failing to meet the requirements of normality were transformed using either square root transformation or logarithmic transformation and retested for normality. Normally distributed phenotypes (following transformation, if necessary) were mapped with parametric linkage analysis (26,27). Traits that were not successfully transformed to normality were analyzed with nonparametric linkage analysis using untransformed data (28). With this methodology, we minimized the risk of high false-positive results associated with linkage analysis of non-normal trait data, as suggested by Kruglyak et al (26,28). Of the six phenotypes measured in the F2 population, only relative left ventricular mass (LVM) was analyzed using nonparametric statistics.

Linkage analysis and QTL identification were performed with MapMaker/EXP to build the genetic maps and MapMaker/QTL for linking the phenotypes to the genetic map, as previously described (29,30). For the parametric linkage test, suggestive and significant of 2.8 and 4.3 were accepted, respectively. For the nonparametric analysis, a threshold of significance was determined at Z score ≥3.5 (27). In a previous study in our laboratory using a similar sized F2 population and almost 20 times the number of measured phenotypes, permutation testing confirmed that these thresholds were appropriate (25).

Analysis of multiple QTL effects

Additional analyses were performed to determine whether the QTLs identified in the above analysis were a result of additive or non-additive interaction effects. MapMaker files (.raw and .maps files) were imported into the rQTL software package (31). Interval mapping was performed in rQTL to confirm the blood pressure QTL identified using MapMaker/QTL. The "scantwo" function within rQTL was then used to test for pairwise effects between all loci. This analysis determines LOD scores for five models between loci as follows: full (additive + epistatic [interaction] effects); interaction; additive; and

conditional-interactive and additive, which compare the interactive and additive effects to a single QTL. Significance thresholds, as suggested for an F2 intercross within the rQTL tutorial, are 9.1, 7.1, 6.3, 6.3, and 3.3 for full, conditional-interactive, interaction, additive, and conditional-additive LOD scores, respectively (31).

Phenotyping for genome scan

All phenotypes for the F2 animals were generated at the University of Otago and published previously as a candidate gene report (19). Briefly, 107 male F2 rats were obtained and the following phenotypes determined at age of 18 ± 1 weeks. Systolic blood pressure (SBP) was measured indirectly in all 107 F2 males using the tail-cuff method, as described elsewhere (18). Two or 3 independent measurements were made over a period of one week and the mean value was calculated. One day after the last tail-cuff measurement, intra-arterial blood pressure and pulse rate were determined directly via a catheter placed in the carotid artery under light anesthesia, after which the rat was killed. Its biometric phenotypes were measured and body weight, LVM, and relative LVM (mg/g body weight) were derived from these measurements.

Phenotyping for single congenic rats

To select the sub-phenotypes (phenotypes hypothesized to be intermediate phenotypes) to be screened in the congenic rats, we used the data set from the PhysGen (*http://pga.mcw.edu*) Program for Genomic Applications. PhysGen had tested the GH and BN (although a different substrain of BN) for 279 heart, lung, and blood traits. Information from this data set guided our decision to challenge the hemodynamic and vasopressor functions of these animals by studying the effects of potent stressors, such as angiotensin II (ANG II) and norepinephrine (NE). Furthermore, we selected several biometrical measurements that were significantly different between the GH and BN in the PhysGen data. These sub-phenotypes were selected because they are potential intermediate phenotypes of blood pressure. Differences in these phenotypes in congenic BN.GH strains encompassing blood pressure QTL would suggest that they may indeed be intermediate phenotypes (32). After preliminary physiological assessment, we noticed that one of the congenic strains (BN.GH18) had a potentially impaired baroreceptor activity. Therefore, to assess baroreceptor sensitivity under the same premise, we also used bolus doses of phenylephrine, as previously described (33).

The following protocol was performed in 16-21 males from each single congenic and parental strains. At the age of 18 ± 1 weeks, microrenathane catheters were implanted in the left femoral artery and vein for blood pressure measurements using the procedure described by Cowley et al (34). After one week of recovery from surgery, catheters were connected to transducers (Argon Medical Technologies, Athens, TX, USA) interfaced with a computerized data acquisition system; then SBP, diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate (HR) were recorded at a frequency of 300 HZ for 4 hours per day for the first three days in the animal's home cage. SBP, DBP, MAP, and HR were averaged over 1-minute periods and converted to a mean daily value for the recording session. The daily averages of MAP, SBP, DBP, and HR for each animal were reduced to a single value for the 3-day recording period. On day 4, we began the challenges using the vasoactive compounds ANGII and NE, both of which were obtained from Sigma (St. Louis, MO, USA) and administered IV using infusion pumps (Harvard Apparatus Inc, Holliston, MA, USA). On day 4, SBP, DBP, MAP, and HR were measured during contin-

uous infusion of ANG II at 4 different doses (day (D)1 = 5 ng/kg/min, D2 = 10″ng/kg/min, D3 = 25 ng/kg/min, and D4 = 50 ng/kg/min) with each dose period of 15 minutes in duration. On day 5, SBP, DBP, MAP, and HR were measured during continuous infusion of NE, at 4 different doses (D1 = 0.1 μg/kg/min, D2 = 0.2 μg/kg/min, D3 = 0.5 μg/kg/min, and D4 = 1.0 μg/kg/min) with each dose period of 15 minutes in duration. SBP, DBP, MAP, and HR were averaged over 1-minute periods and converted to a mean 15-minute value for the recording session for each dose of ANGII and NE. This protocol was previously used and published (*http://pga.mcw.edu*). On day 6, arterial baroreceptor sensitivity was determined by modified Oxford method (33), recording SBP and HR first 30 seconds after IV bolus doses of phenylephrine (0.1 ml, 4 μg/kg), obtained from Sigma.

On the last day of the experiment, animals were killed with CO_2 and organs (kidney, heart) were harvested and weighed. Body weight was also measured.

Measurement of blood pressure in double and triple congenics

Blood pressure was measured in BN, GH, and double and triple congenic strains by radiotelemetry using the Dataquest IV system (Data Sciences Inc, St. Paul, MN, USA). Rats were anesthetized with 1% isoflurane and a femoral catheter was implanted in the left femoral artery. The telemetry catheter is attached to a TA11PA-C40 radio-telemetry transmitter, which was placed subcutaneously in the back of the rat. Rats were placed in individual cages after 3 days of recovery from surgery; blood pressure was measured for three days during a low salt diet (0.4% NaCl). Rats where then switched to a 4% NaCl diet and blood pressure was measured every other day for 23 days. Blood pressure was measured for 10 seconds every two minutes for a total of four hours on each experimental day and averaged after discarding the first 30 minutes of recording. The daily averages of blood pressure were reduced to a single value for the recording period.

Statistical analysis

All data are presented as mean ± standard deviation unless stated otherwise. Baseline SBP, DBP, MAP, and HR data were evaluated by analysis of variance (ANOVA) with a post-hoc analysis using Dunnett test (multiple comparisons vs control BN group) (SigmaStat, version 2.03; SPSS Inc., Chicago, IL, USA).

Vascular reactivity data (NE and ANG II) were fit to linear regression equations: $VR = \alpha_{0+} \beta[x]$, where VR is vascular reactivity, α_0 is an intercept term, [x] is drug concentration, and β is slope coefficient. The *t* statistic for the slope was significant at the 0.05 critical α level. For all regression equations, r^2 was >0.80, ie, it explained more than 80% of the variance in the dependent variable by the regression equation. We defined β (slope) coefficient as the reactivity index representing the change in mean blood pressure for an incremental change in the independent variable (NE and ANG II concentration), as previously described (35,36).

Heart rate response to SBP changes (a measure of baroreflex sensitivity) caused by phenylephrine bolus doses was recorded and linear regression analysis was performed, whereby slope coefficients (β) of each animal's regression line were averaged and taken as an index of baroreceptor sensitivity (33). NE, ANG II, baroreceptor sensitivity, and slope coefficients were separately evaluated by analysis of variance (ANOVA) followed by Dunnett test. In all cases, a value of $P \leq 0.05$ was considered to be statistically significant. All statistical analyses were performed with SigmaStat, version 2.03 (SPSS Inc., Chicago, IL, USA)

Results

Genome scan results

The genome size in this cross was calculated to be 1412.4 cM, consistent with previously published maps obtained in other linkage analysis studies using male F2 populations and ranging from 1509 cM (37) to 1749 cM (38) to 1831 cM (39). In our study, genome coverage averaged every 5.4 cM. In total, 8 QTL were assigned to the genetic map (five significant with LOD score ≥4.3 and three suggestive with LOD≥2.8), corresponding to five of six measured phenotypes (tail blood pressure, intra-arterial blood pressure, body weight, LVM, and relative LVM) (Table 1). Overlapping QTL regions for blood pressure mapped to chromosomes 2, 6, and 18 were identified in other crosses involving other hypertensive strains, suggesting that these loci are likely to exhibit true linkage. The QTL for blood pressure on chromosome 6 was identified by both the direct and indirect measurements of blood pressure, further supporting the existence of a blood pressure regulating gene(s) at this location. Furthermore, body weight was mapped to a region of chromosome 3 (LOD 5.2); LVM was mapped to chromosomes 3 and 13 (LOD 4.6 and 4.1, respectively), while relative LVM was mapped to chromosome 10 only (LOD 4.6). Heart rate was not mapped in this cross.

Additive and epistatic QTL

Table 2 displays the results from the scan two analysis for the tail-cuff blood pressure QTL on chromosomes 2, 6, and 18. All pairwise comparisons showed significant full LOD scores, which reflect both additive and interactive effects. However, there is no evidence for significant pairwise interactions for any of the loci, although a non-significant trend for interaction was seen between chromosomes 2/18 and 2/6. Moreover, there is significant evidence for additive effects for all three pairwise comparisons. From this data, we hypothesized that single congenic strains should significantly affect blood pressure.

Results from studying the congenics

Genome characterization. The blood pressure QTL were followed up by generating three congenics, in which each blood pressure QTL from GH was introgressed onto the genome background of the normotensive BN (Figure 1). In each case, we attempted to capture the

Table 1. Quantitative trait loci (QTL) mapped in an F2 intercross between Genetically Hypertensive (GH) and Brown Norway (BN) rats

No.	Traits	Chromosome No.	Flank1	Flank2	LOD*	Size (cM)	Peak
1	Tail cuff blood pressure (mm Hg)	2	*D2Mgh7*	*D2Mgh12*	3.58†	25.8	*D2Mgh8*
2	Tail cuff blood pressure (mm Hg)	6	*D6Mit12*	*D6Mit3*	4.30‡	73.4	*D6Mit9*
3	Tail cuff blood pressure (mm Hg)	18	*D18Mgh4*	*D18Mgh2*	4.05†	19.5	*D18Mgh6*
4	Left ventricle mass (mg)	3	*D3Mgh21*	*D3Mgh2*	4.63‡	48.4	*D3Mit4*
5	Left ventricle mass (mg)	13	*D13mgh13*	*D13Kid1*	4.12†	34.6	*D13Arb7*
6	Direct blood pressure (mm Hg)	6	*D6Pas1*	*D6Mit3*	4.80‡	71.9	*D6Mit9*
7	Body weight (g)	3	*D3Mgh21*	*D3Mgh2*	5.19‡	48.4	*D3Mit4*
8§	Relative left ventricle mass (mg/g)	10	*D10Mit6*	*D10Mit7*	4.55‡	22.5	*D10Mgh8*

*LOD – $\log_{10}$ of the odds.
†Suggestive linkage.
‡Significant linkage.
§Mapped with nonparametric linkage analysis.

Table 2. Analysis of blood pressure quantitative trait loci (QTL) for additive and interacting effects*

Locus 1 (RNO; cM position)*	Locus 2 (RNO; cM position)	LOD full	LOD conditional-interactive	LOD Interaction	LOD additive	LOD conditional-additive
6; 34	18; 15	9.86†	5.08	0.72	9.13†	4.35†
2; 42	18; 14	9.37†	5.58	1.70	7.67†	3.88†
2; 42	6; 33	9.50†	4.72	1.83	7.67†	2.89

*Abbreviations: RNO – rat chromosomes, LOD – log10 of the odds.
†Significant, based upon 10 000 simulations of crosses with 250 individuals, markers at a 10 cM spacing, and analysis by Haley-Knott regression (31).

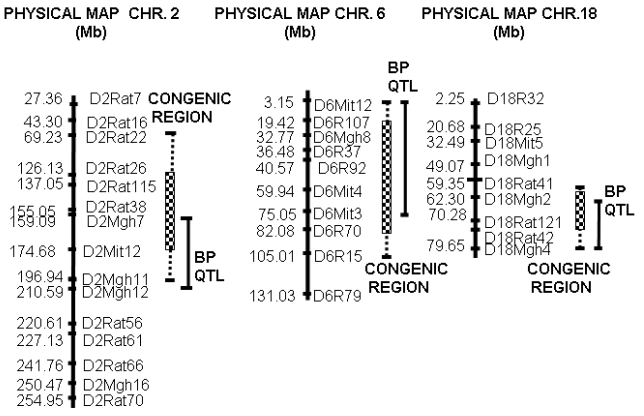

Figure 1. Genetic maps superimposed on the genomic sequence of rat chromosomes 2, 6, and 18 with blood pressure quantitative trait loci (BP QTL), congenic regions, and flanking markers. Checker board filled lines represent introgressed BP QTL regions, horizontal bars are flanking markers used to construct the congenics. It is important to note that these congenics were generated long before there was genomic sequence to firmly establish genetic order or genetic distances. The brackets denote the mapped QTL (99% confidence interval).

99% confidence interval. To confirm the congenics were isogenic, we scanned the genome with 112 polymorphic markers (~1 marker per 20 cM). All markers were homozygous for BN, demonstrating that passenger loci from the GH are likely to be few. All QTL encompassed a large chromosomal interval, based on the genomic sequence of the rat: 127.71 Mb for chromosome 2, 101.86 Mb for chromosome 6, and 20.3 Mb for chromosome 18; consequently, the congenic regions were scanned more densely, approximately one marker every 8-10 cM. For the BN.GH6 and BN.GH18 congenics, we captured the complete QTL confidence interval. For the BN.GH2 congenic, 14 Mb was excluded due to a misplaced marker in previous genetic maps. The double congenic and triple congenic animals had the same introgressed regions as the single congenic animals.

Baseline blood pressure

Initially, five groups of animals (GH, BN.GH2, BN.GH6, BN.GH18, and BN) were studied. Despite carrying a QTL for hypertension, none of the blood pressure or heart rate phenotypes in any of the three single congenics differed from the BN (Table 3). GH rats had significantly higher MAP than BN and all of the single congenics strains. Consequently, transferring a single QTL from the hypertensive GH onto the normotensive BN strain was not sufficient to cause a significant increase in baseline blood pressure. This could be due to genetic factors on the BN genome that are highly resistant to hypertension, due to epistatic relationships between blood pressure QTL, or due to a threshold effect (ie, multiple QTL are required to overcome homeostasis on a resistant genome background). Because the QTL analysis did not indicate significant interactions between QTL, we hypothesized there was a possible threshold effect and, therefore, measured blood pressure in double and triple congenics using telemetry monitoring. Unfortunately, none of the double or the triple congenic strains was sufficient to significantly increase blood pressure on the BN background, even after exposure to a high salt diet (Table 4).

Biometric measurements

To assess end organ damage in the congenics, heart and kidney weights (absolute and normalized by body weight) were determined in the single congenic and parental strains (Table 5). It is not surprising that the congenics showed no differences in kidney measures as they also did not significantly differ between BN and GH. Although the GH have a significantly higher absolute and adjusted heart weight than BN, we did not observe any significant differences in the congenics compared with the BN parental control, indicating that hypertrophy requires hypertension as a driver or may be under different genetic control than blood pressure in the GH. However, independent genetic factors causing cardiac hypertrophy would not necessarily be surprising, as the QTL for LVM and left ventricular hypertro-

Table 3. Baseline mean arterial pressure, systolic blood pressure, diastolic blood pressure, and heart rate values in single congenic and parental strains*

	Rat strain (mean ± standard deviation)				
Trait	GH (n = 21)	BN.GH2 (n = 19)	BN.GH6 (n = 16)	BN.GH18 (n = 21)	BN (n = 21)
Mean arterial pressure (mm Hg)	155.7 ± 14†	103.8 ± 9.2	97.3 ± 11	98.0 ± 6.7	99.8 ± 10.0
Systolic blood pressure (mm Hg)	190.9 ± 9.7†	124.6 ± 7.4	114.4 ± 9.5	116.7 ± 6.9	116.4 ± 7.8
Diastolic blood pressure (mm Hg)‡	129.5 ± 15†	86.9 ± 9.7	82.7 ± 12	82.6 ± 8.3	85.3 ± 12.0
Heart rate (beats per minute)	365.0 ± 23.0	349.8 ± 32.0	381.3 ± 32.0	361.7 ± 33.0	362.0 ± 32.0

*Abbreviations: GH – Genetically Hypertensive rats; BN – Brown Norway rats; BN.GH2 – BN.GH-(*D2Rat22-D2Mgh11*)/Mcwi; BN.GH6 – BN.GH-(*D6Mit12-D6Rat15*)/Mcwi; and BN.GH18 – BN.GH-(*D18Rat41-D18Mgh4*)/Mcwi
†$P < 0.05$ vs BN. All data were evaluated by analysis of variance (ANOVA) with a post-hoc analysis using Dunnett test (multiple comparisons vs control BN group).
‡Measured using an intra-arterial catheter.

Table 4. Mean arterial pressure in double and triple congenic and parental rat strains

	Rat strain (mean ± standard deviation)					
Trait	GH (n = 20)	BN.GH2.6 (n = 16)	BN.GH6.18 (n = 16)	BN.GH2.18 (n = 16)	BN.GH2.6.18 (n = 16)	BN (n = 21)
Mean arterial pressure (mm Hg) on 4% NaCl diet	147.5 ± 9.2†	98.1 ± 7.9	99.5 ± 10.4	110.1 ± 7.8	93.5 ± 8.6	93.0 ± 9.3

*Abbreviations: GH –Genetically Hypertensive rats; BN – Brown Norway rats; BN.GH2 – BN.GH-(*D2Rat22-D2Mgh11*)/Mcwi; BN.GH6 – BN.GH-(*D6Mit12-D6Rat15*)/Mcwi; and BN.GH18 – BN.GH-(*D18Rat41-D18Mgh4*)/Mcwi.
†$P < 0.05$ vs BN, all values were calculated by analysis of variance (ANOVA) with a post-hoc analysis using Dunnett test (multiple comparisons vs control BN group).

Table 5. Biometric measurements in single congenics and parentals*

	Rat strain (mean ± standard deviation)				
Trait	GH (n = 21)	BN.GH2 (n = 19)	BN.GH6 (n = 16)	BN.GH18 (n = 21)	BN (n = 21)
Kidney weight (g)	1.22 ± 0.05	1.15 ± 0.15	1.12 ± 0.2	1.17 ± 0.2	1.12 ± 0.10
Adjusted kidney weight per 100 g of body weight (g/100 g)	0.34 ± 0.02	0.37 ± 0.03	0.37 ± 0.04	0.38 ± 0.04	0.36 ± 0.04
Left ventricular mass (g)	1.69 ± 0.13†	1.13 ± 0.15	1.17 ± 0.23	1.15 ± 0.20	1.15 ± 0.13
Left ventricular hypertrophy (cardiac hypertrophy) per 100 g of body weight (g/100 g)	0.47 ± 0.07†	0.34 ± 0.05	0.38 ± 0.03	0.37 ± 0.03	0.37 ± 0.06
Body weight (g)	365.4 ± 17.0†	330.6 ± 26.0	299.9 ± 39.0	315.2 ± 24.0	310.3 ± 35.0

*Abbreviations: GH – Genetically Hypertensive rats, BN – Brown Norway rats; BN.GH2 – BN.GH-(*D2Rat22-D2Mgh11*)/Mcwi; BN.GH6 – BN.GH-(*D6Mit12-D6Rat15*)/Mcwi; and BN.GH18 – BN.GH-(*D18Rat41-D18Mgh4*)/Mcwi.
†$P < 0.05$ vs BN. All data were evaluated by analysis of variance (ANOVA) with a posthoc analysis using Dunnett test (multiple comparisons vs control BN group).

Table 6. The β coefficients (slope) of blood pressure responses to angiotensin II and norepinephrine in single congenic and Brown Norway (BN) negative controls*†

	β coefficients in rat strain (mean ± standard deviation)				
Drug	GH (n = 14)	BN.GH2 (n = 11)	BN.GH6 (n = 11)	BN.GH18 (n = 11)	BN (n = 14)
Norepinephrine	0.34 ± 0.10‡	0.28 ± 0.9	0.19 ± 0.8	0.26 ± 0.11	0.28 ± 0.05
Angiotensin II	0.48 ± 0.08‡	0.66 ± 0.09‡	0.86 ± 0.08	0.71 ± 0.07‡	0.88 ± 0.08

*Abbreviations: GH – Genetically Hypertensive rats; BN – Brown Norway rats; BN.GH2 – BN.GH-(D2Rat22-D2Mgh11)/Mcwi; BN.GH6 – BN.GH-(D6Mit12-D6Rat15)/Mcwi; and BN.GH18 – BN.GH-(D18Rat41-D18Mgh4)/Mcwi.
†The β coefficients (slope) in the congenics and the parental rats are estimates of the vascular responsiveness: the higher the β coefficient, the greater the rate of change in response to the vasoactive agent.
‡$P < 0.05$ vs BN. All data were evaluated by analysis of variance (ANOVA) with a post-hoc analysis using Dunnett test (multiple comparisons vs control BN group).

phy were mapped to different loci in the F2 intercross.

NE and ANGII challenge

The cardiovascular system of the rats was challenged with NE and ANG II to identify additional sub-phenotypes (phenotypes hypothesized to be intermediate phenotypes of hypertension). Animals were tested with four different doses of each vasoconstrictor, ranging from 0.1 to 1.0 μg/kg/min (NE) and from 5 to 50 ng/kg/min (ANG II). The changes in mean arterial pressure caused by administration of NE and ANG II were fitted to linear regression equations and we calculated β coefficients (slope) for each of strains (11-14 rats in each group). The β coefficients (slope) of blood pressure responses to NE and ANG II are summarized in Table 6. We did not find any differences in the β coefficient after administration of NE (Figure 2). However, two congenic strains, BN.GH2 and BN.GH18

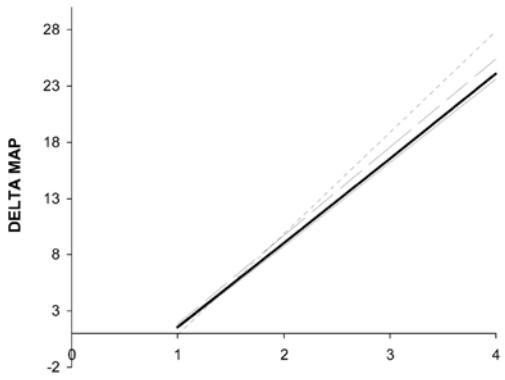

Figure 2. Blood pressure response to norepinephrine (NE) x-axis shows different concentration of administered drugs as follows: 1 = 0.1 μg/kg/min, 2 = 0.2 μg/kg/min, 3 = 0.5 μg/kg/min, and 4 = 1.0 μg/kg/min. y-axis shows calculated mean delta mean arterial pressure value between 10th and 15th minute of infusion. BN.GH2 (n = 11) – doted line, BN.GH6 (n = 11) – full line, BN.GH18 (n = 11) – dasehs line, and BN (n = 14) – bold line. Lines represent linear regression lines (slopes) for each group. All data were evaluated by analysis of variance (ANOVA) with a post-hoc analysis using Dunnett test (multiple comparisons vs control BN group).

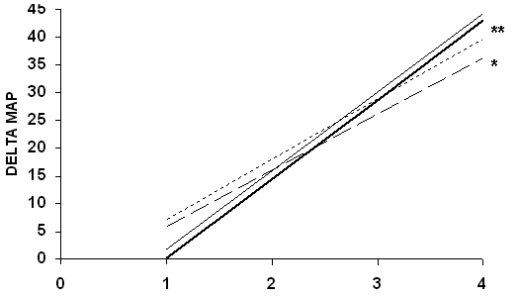

Figure 3. Blood pressure response to angiotensin II (ANGII). x-axis shows different concentration of administered drugs as follows: 1 = 5 ng/kg/min, 2 = 10 ng/kg/min, 3 = 25 ng/kg/min, and 4 = 50 ng/kg/min. y-axis shows calculated mean delta mean arterial pressure (MAP) value between minutes 10 and 15 of infusion. BN.GH2 (n = 11) – dashed line, BN.GH6 (n = 11) – full line, BN.GH18 (n = 11) – doted line, and BN (n = 14) – bold line. Lines represent linear regression lines (slopes) for each group. MAP responses to ANGII were significantly enhanced in BN.GH2 and BN.GH18 rats compared with control. $P < 0.05$ BN.GH2 vs BN; $P < 0.05$ BN.GH18 vs BN. All data were evaluated by analysis of variance (ANOVA) with a post-hoc analysis using Dunnett test (multiple comparisons vs control BN group).

(Figure 3), had significant differences in their blood pressure response to ANG II than that of the parental BN strain, indicating that these congenic strains may harbor a gene(s), which affect their response to this vasoconstrictor.

Baroreceptor reflex sensitivity

After analyzing heart rate response to ANG II, we found that only one congenic strain, BN.GH18, had a response different from BN, which was consistent with previous findings of baroreceptor QTL on chromosome 18 in SS rats (25). This finding prompted us to test baroreceptor sensitivity using bolus doses of phenylephrine (IV) in BN.GH18 and parental controls. Phenylephrine was given to produce sudden blood pressure increases and the resulting decreases in heart rate were measured as previously described (33). Linear regression analysis was used to calculate the β coefficients, which is a common index of baroreceptor sensitivity. BN.GH18 (N = 11) rats revealed significantly lower baroreceptor sensitivity compared with the BN (N = 14) normotensive control ($\beta = -1.25 \pm 0.17$ vs $\beta = -2.28 \pm 0.26$); $P < 0.005$). This baroreceptor reflex activity was also abnormal in GH (N = 14) compared with BN rats ($\beta = -0.87 \pm 0.25$, $P < 0.001$), suggesting that absence or impairment of the reflex may be an important blood pressure subphenotype mapped to the chromosome 18 QTL.

Discussion

The present study represents a first complete genome-wide scan for linkage to hypertension and hypertension-induced end-organ damage in the GH rat strain. While associations between some cardiovascular phenotypes and several candidate gene alleles (*GCA, REN, TNF α*) in GH rats have already been reported in a GH × BN intercross (19,40,41), we report, for the first time, all data and results of the complete genome scan including QTL for five of the six measured or derived traits on six different chromosomes. Three major blood pressure QTL in the GH rat were identified (1 significant QTL on chromosome 6 and 2 suggestive on chromosomes 2 and 18). Furthermore, we mapped two QTL for LVM, one concurrent with a

body weight QTL, and one QTL for left ventricular hypertrophy.

To follow up the blood pressure QTL, we initially constructed single congenic strains for each of those three regions, introgressing the susceptible GH allele onto the genome background of the resistant BN rat. Overall, the phenotypic data showed that the transfer of any one of the QTL regions (chromosome 2, 6, or 18) from GH onto BN background was not sufficient to independently induce hypertension. A likely explanation of our results is the protective phenotypic effect of the BN genome background in the presence of a hypertension QTL. Although it is possible that the initial linkage analysis captured weak QTL effects (since QTL on chromosomes 2 and 18 had only suggestive LOD scores (LOD 3.6 and 4.1, respectively), it is unlikely that all three loci would be false positives given that the linkage data identified a QTL on chromosome 6 above the significance threshold of LOD 4.2 both by indirect and direct blood pressure measurements; that the pairwise analysis indicated significant LOD scores for chromosomes 2, 6, and 18; and that the literature reports several coinciding blood pressure QTL in different F2 intercrosses on chromosomes 2 (SHR × WKY; SHR × BN; SS × BN; LN × LH; S × WKY; S × MNS; SS × WKY; SHRSP × WKY) (18,42-47), chromosome 6 (SS × BN) (25), and chromosome 18 (SHR × BB/OK and BN × SS) (25,48,49). Although possible, it is also unlikely that the shorter congenic interval introgressed in the BN.GH2 resulted in protection from hypertension.

The approach of transferring a "disease" allele onto a "normal" background has worked in some instances and not in others (50,51). Likely, this is due to background strain differences. For instance, Bianchi and Tripodi (51) transferred a hypertension QTL from the MHS onto the normotensive MNS background and observed a significant blood pressure increase. However, these strains are genetically much more similar than are GH and BN (62% similar vs 28% similar, respectively, according to genotype information of nearly 4500 SSLP markers) (*http://rgd.mcw.edu/strains/poly_table0bp.shtml*) (52); it is quite likely that they share similar modifiers or additional QTL as well. However, given different strains, we and others have found that transferring a single disease locus onto a resistant background is sometimes not sufficient to trigger the onset of polygenic diseases, such as hypertension, due to epistatic interaction with other QTL or due to multiple genetic modifiers present in the hypertensive strain (53,54). The advantage of introgressing a disease allele onto a "normal" background allows the exclusion of the influence of a susceptible genome background to explain individual effect of gene(s) in the regions of interest. This question cannot be addressed by the reciprocal congenic schema, ie, introgressing a normal allele into a susceptible genome background. Initial findings prompted us to test our hypothesis that more than one QTL is required to affect blood pressure in the congenic GH.BN congenics, by generating and characterizing double and triple congenic strains, again without confounding background effects. Unfortunately, we were not able to detect any blood pressure effects in the double and triple congenic strains. Potentially the BN is not normotensive, but hypertension resistant. Therefore, even three significant QTL from the GH rat, introgressed onto the BN genome, are insufficient to overcome the homeostatic pressures in the BN rat. The congenics we developed could be used to test this hypothesis by performing an F2 intercross between the congenic (single, double, or triple) strains and the GH rat. This approach would allow us to map "resistance genes" in the BN background or modifier genes in the GH ge-

nome, while keeping the blood pressure QTL fixed for the GH allele.

Our study design also allowed us to ask if we could detect phenotypic differences in putative intermediate phenotypes in the absence of a change in blood pressure. Our hypothesis predicted we would find sub-phenotypes related to blood pressure regulation that would be altered in the congenic animals, even though they were all normotensive. To test this, we challenged the single congenic and parental strains pharmacologically using NE and ANGII, each of which increases blood pressure, but by different mechanisms. We found that, despite normal levels of blood pressure, some of our congenics showed different responses to the vasopressor actions of ANGII, suggesting it could be an intermediate phenotype in the BN.GH2 and BN.GH18. Since we previously mapped the baroreceptor reflex to chromosome 18 (25), although in a different rat strain (SS rat), we decided to test only BN.GH18 congenic for baroreceptor sensitivity because of the reduced heterogeneity as compared with an F2 intercross between SS and BN. This study showed that BN.GH18 rats had a significantly lower baroreceptor reflex response, compared with normotensive BN controls, suggesting that we may have captured a major locus responsible for altered baroreceptor sensitivity. Several studies suggest that baroreflex function is strongly influenced by genetic factors (55,56), but the level of the genetic contribution is not completely known (57). Given that many studies suggest that baroreflex abnormalities are an important contributing factor to the pathogenesis of hypertension (58-62), we consider this result to suggest that baroreceptor sensitivity can be considered a sub-phenotype in the BN.GH18 rat strain, not secondary to hypertension itself. This baroreceptor sensitivity may contribute to the development of hypertension if placed in a susceptible background, but it may be compensated in the BN background.

In summary, our findings suggest that, for complex diseases such as hypertension, gene(s) from one candidate region may not be sufficient to independently cause a complex phenotype and that genomic background also plays a significant and often ignored role. When dealing with complex phenotypes such as hypertension, analysis of multiple loci, related potential intermediate phenotypes, and their interactions may help us to understand pathways involved in their homeostasis. Despite the fact the single and multiple locus congenic rats did not show any alterations in blood pressure by isolating hypertension susceptible (GH) QTL on a normotensive background (BN), we were able to determine likely intermediate phenotypes within the QTL that influence hypertension on chromosomes 2 and 18 and to confirm the presence of genetic determinants of the baroreceptor reflex on chromosome 18. Our future steps will include further evaluation of these genome regions, selection of candidate genes followed by gene expression, and physiological studies.

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