# Contribution of Autosomal Loci and the Y Chromosome to the Stress Response in Rats

Pierre Dumas, Zdenka Pausová, Vladimír Kren, Drahomíra Krenová, Michal Pravenec, Marc Dumont, Daniel Ely, Monte Turner, Yulin Sun, Johanne Tremblay, Pavel Hamet

***Abstract***—Stress is a critical contributor to cardiovascular diseases through its impact on blood pressure variability and cardiac function. Familial clustering of reactivity to stress has been demonstrated in human subjects, and some rodent models of hypertension are hyperresponsive to stress. Therefore, the present study was designed to uncover the genetic determinants of the stress response. We performed a total genome linkage search to identify the loci of the body temperature response to immobilization stress in a set of recombinant inbred strains (RIS) originating from reciprocal crosses of spontaneously hypertensive rats (SHR) with a normotensive Brown Norway Lx strain. Two quantitative trait loci (QTLs) were revealed on chromosomes (Chrs) 10 and 12 (logarithm of odds scores, 2.2 and 1.3, respectively). The effects of these QTLs were enhanced by a high sodium diet (logarithm of odds scores, 4.0 and 3.3 for Chrs 10 and 12, respectively), which is suggestive of a salt-sensitive component for the phenotype. Congenics for Chr 10 confirmed both the QTL and the salt effect in RIS. Negatively associated loci were also identified on Chrs 8 and 11. Interaction between the loci of Chrs 10 and 12 was demonstrated, with the rat strains bearing SHR alleles at both loci having the highest thermal response to stress. Furthermore, the Y Chr of SHR origin enhanced the response to immobilization stress, as demonstrated in 2 independent models, RIS and Y Chr consomics. However, its full effect requires autosomes of the SHR strain. These findings provide the first evidence for the genetic determination of reactivity to stress with interactions between autosomal loci and between the Y and autosomal Chrs that contribute to the explanation of the 46% of variance in the stress response. **(*Hypertension*. 2000;35:568-573.)**

**Key Words:** stress ■ linkage (genetic) ■ quantitative trait ■ sex chromosomes



The response to environmental stress is a predictor of cardiovascular diseases, including future high blood pressure (BP), in prehypertensive patients with a positive family history[1] and the development of left ventricular hypertrophy.[2] Spontaneously hypertensive rats (SHR) and mice are more reactive to stress than their normotensive counterparts.[3–6] On exposure to several psychogenic stressors, such as cage-switch, placement in an open field, or immobilization, they display higher changes in heart rate, BP, and body temperature (BT).[5,7] The increase in BT with immobilization stress may, thus, be proposed as an intermediate phenotype of the stress response. To identify the genetic determinants of the BT response to immobilization stress, we performed a full genome scan in a set of rat recombinant inbred strains (RIS) originating from reciprocal crosses of SHR and normotensive Brown Norway (BN) Lx strains. This is the only set of rat RIS available to study the genetics of hypertension and its related traits.[8] Any quantitative phenotype that can be measured accurately and that displays a significant gradient across strains can be mapped in the RIS panel, irrespective of the differences between progenitor strains,[9] in this permanent replica of the $F_2$ generation rendered homozygous by over 30 generations of inbreeding. Therefore, we used this paradigm to identify quantitative trait loci (QTL) of the stress response variance with increased BT after immobilization as a phenotype. Because our previous data indicated that high sodium intake heightens the response to immobilization stress,[10] we measured BT in rats fed normal and high sodium diets.

## Methods

### Rats

Male rats weighing 150 to 300 g were studied. RIS reciprocal crosses were derived from SHR/Ola and BN.Lx/Cub strains, as described previously.[8] BN.Lx rats are congenic rats carrying a segment of chromosome (Chr) 8 from polydactylous (PD)/Cub strains.[11] They are genetically distant from SHR and were thus chosen for the breeding and development of segregating strains because more polymorphic markers can be found between the 2 strains.[8] Experiments were performed during 4 separate time periods in both Prague and Montreal, which enabled us to test many of the strains twice

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From the Centre de Recherche du CHUM, Université de Montréal, Montréal, Québec, Canada (P.D., Z.P., M.D., Y.S., J.T., P.H.); the Institute of Biology, First Medical Faculty, Charles University, Prague, Czech Republic (V.K., D.K., M.P.); the Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic (V.K., M.P.); and the Department of Biology, University of Akron, Akron, Ohio (D.E., M.T.).

Correspondence to Johanne Tremblay or Pavel Hamet, Laboratory of Cellular Biology of Hypertension and Laboratory of Molecular Medicine, Centre de Recherche-CHUM, 3850 Rue Saint-Urbain, Montréal, Québec, Canada H2W 1T8. E-mail hamet@ere.umontreal.ca



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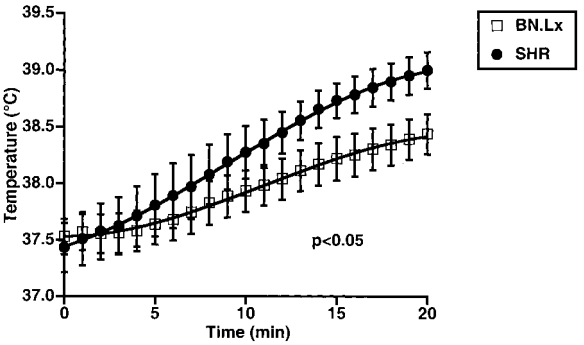


**Figure 1.** BT during immobilization stress in BN.Lx and SHR strains. Values are mean±SEM. $P<0.05$ by ANOVA; n=8 and n=5 for BN.Lx and SHR, respectively.

under both dietary conditions to confirm the phenotypic values obtained. The rats were maintained on a 12-hour day/night cycle at a room temperature of 23 to 25°C. They were given access to water and food ad libitum, and the protocols of the study were approved by the local animal care committees.

A total of 27 RIS were used for stress-response phenotyping. The high Na diet consisted of normal rat chow (0.6% Na) supplemented with NaCl to raise the Na content to 3.15% by weight (8% NaCl). Y Chr consomic strains, originally designated as SHR/y and SHR/a, were developed from Wistar-Kyoto (WKY) and SHR inbred colonies kept at the University of Akron, Ohio, as described previously.[12] For uniformity of nomenclature, we designated them here as WKY.SHR-Y and SHR.WKY-Y, respectively, according to their somatic and sexual Chr origins.

The SHR.BN-Y consomic strain was produced by introgressing the Y Chr from BN males into the SHR genetic background (V. Kren, DSc, et al, unpublished data, 1998). The BN Y Chr is cytogenetically distinguishable from the Y Chr of SHR origin (M. Sladká, oral communication, 1998). SHR.BN-*Rattus norvegicus* Chr 10 (RNo10) congenics were obtained by introgressing a segment of BN Chr 10 onto the SHR genetic background. After 9 backcrosses, an intercross was performed, and *Myh3* BB homozygotes were selected by polymerase chain reaction genotyping. *Myh3* BB–homozygous congenic males from *N9F3* with residual heterozygosity in *Myh3* flanking markers were used. *D10Wox11*, *D10Mit4*, and *D10Rat31* markers were BB homozygotes. Residual heterozygosity was observed for the following *Myh3* flanking markers: *D10Wox12*, *D10Wox14*, *D10Mit2*, *D10 Mgh6*, *D10Rat59*, and *D10Rat102*. *D10Mit1*/*Ace*, *D10Mit5*, and *D10Mit7* were HH homozygotes (D. Krenova, DSc, et al, unpublished results, 1998). A total of 2 to 12 rats per RIS were used for phenotyping. For congenic and consomic strains, 6 to 9 rats were used (see Figures).

## Thermal Response to Stress

BT after immobilization served as a reproducible marker of the stress response. To control the variation of the circadian rhythm of BT, all measurements were conducted between 8:00 and 12:00 AM in a calm environment. Naive animals were placed in transparent plastic restraint holders routinely used for tail-cuff determination of BP in rats, and a rectal thermal probe was introduced (3 to 5 cm) rapidly and secured around the tail. Rectal temperature was monitored continuously during the 20-minute immobilization period (T20). Two rats could be recorded simultaneously, with randomization of temperature probes and strains. Temperature thermistors with a linearity between 5 and 45°C and a precision of 0.1°C were used (YSI 44202, Yellow Springs Instrument Co).

## Genetic Analysis

The heritability of T20 under both dietary conditions was estimated using the following formula: heritability=Va/(Va+Ve), where Va is the interstrain variance and Ve, the intrastrain variance. Strain distribution patterns of the thermal response to stress observed on high and normal Na diets were correlated with those of 475 biochemical, morphological, immunogenetic, and molecular genetic markers available from the ratmap Internet site.[13] The nomenclature and descriptions are reported in detail elsewhere.[9,14] The linkage map of RIS covers 1139 centimorgans (cM) of the rat genome, with a mean of 20 markers per Chr, as described previously.[14]

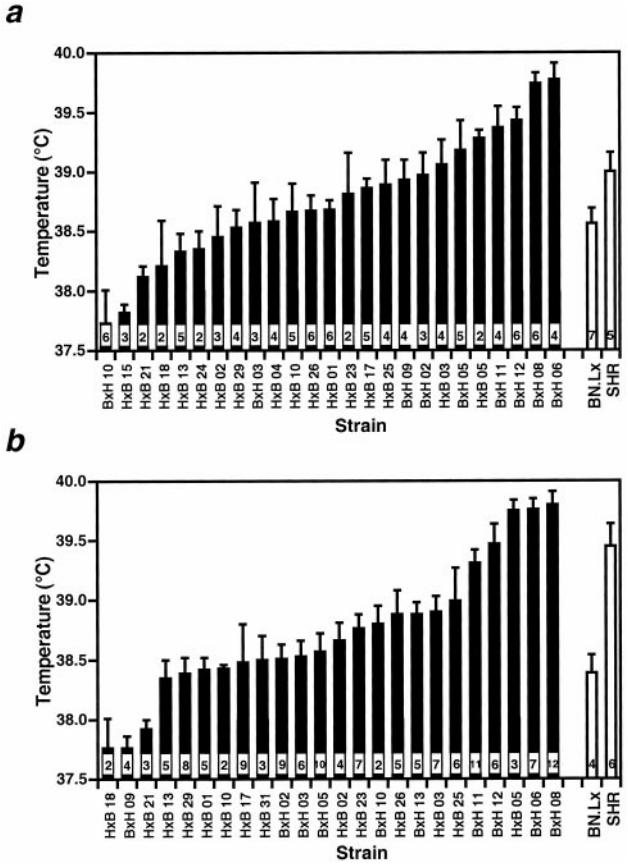


**Figure 2.** a, Strain distribution pattern for T20 (mean±SEM) measured under normal Na intake in 25 RIS. b, Strain distribution pattern of T20 measured under high Na intake in 24 RIS. White bars indicate BN.Lx and SHR progenitor strains. B×H are strains originating from crosses of female BN.Lx and male SHR, whereas H×B are strains originating from crosses of female SHR and male BN.Lx. Shown within bars are number of rats used per strain.

## Total Genome Linkage Search

Linkage analysis was performed with MapManager software, which was developed and adapted by Dr K.F. Manly for QTL analysis (MapManager QT v3.0b21).[15,16] Because of multiple simultaneous comparisons, stringent statistical criteria were used to avoid false-positive linkages.[9] We ran a nonparametric permutation test, as proposed by Doerge and Churchill[17] and implemented on MapManager, which estimates thresholds of findings specified by linkage statistics on total genome scan. The thresholds used were 0.5, 0.05, and 0.001, which corresponded to suggestive, significant, and highly significant linkages, respectively.[18] The permutation test was run for each phenotype every 5 cM with 500 permutations to simulate the experimental conditions of marker density and multiple comparisons required, respectively. Pearson product-moment correlation analysis, which is equivalent to the Holm's *t*-test, was also used. It provided signs of association and an estimate of the percentage of variance of the trait explained by the locus ($r^2$).

## Statistics

Data are presented as the mean±SEM obtained for a single measurement in several animals, as indicated in Results. We evaluated

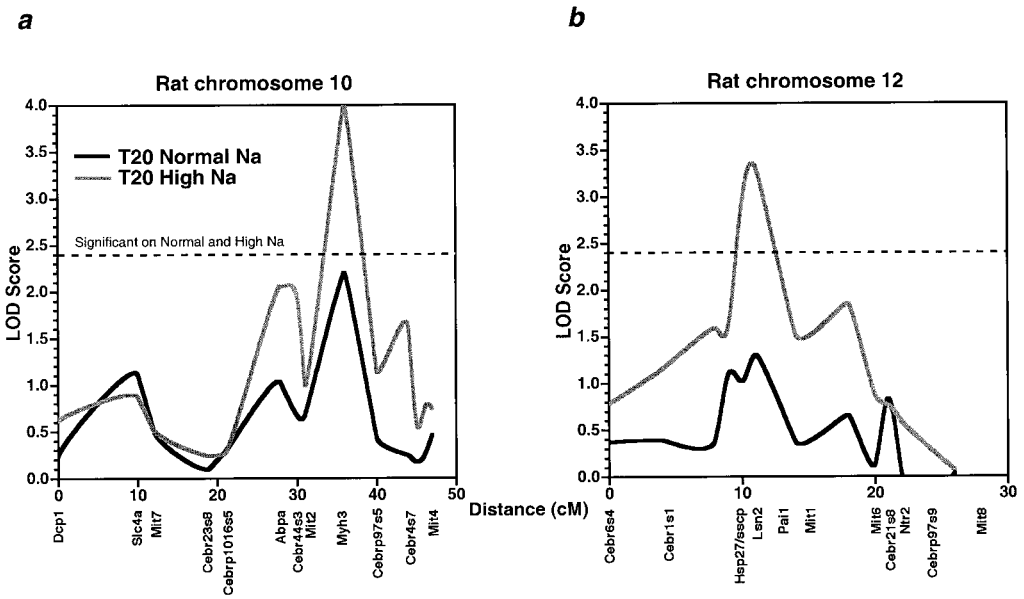


**Figure 3.** QTL plot for T20 measured in 21 RIS under normal and high Na intake. Dotted line indicates significance threshold according to permutation test. The 95% confidence intervals (CI) were calculated according to the method of Darvasi and Soller[19] using the following formula: CI=530/(n×$r^2$), where n=49 strains and $r^2$=proportion of variance explained. Data (strains) from both conditions were combined. a, Chr 10. Partial linkage map from Dcp1 to Mit4 from Pravenec et al[14] Chr designation (D10) is omitted. CI=26 cM. b, Chr 12. Partial linkage map from Cebr6s4 to Mit8 from Pravenec et al[13] Chr designation (D12) is omitted. CI=33 cM.

the relative contribution of each significant locus with a multiple linear regression model. The dependent variable was T20, and the independent predictors were the individual significant loci taken as dichotomous variables (−1 for BN.Lx genotypes; 1 for SHR genotypes). The diet effect was also studied (−1 for normal diet; 1 for the high-salt diet). All analyses were considered significant at the 0.05 $\alpha$ level. Data from individual rats were computed.

## Results

Although the basal BT of SHR and BN.Lx strains was identical, immobilization stress for 20 minutes induced a greater thermal response in SHR compared with BN.Lx, as illustrated by a higher temperature and a higher rate of BT increase (Figure 1). Rectal temperature at T20 served as the quantitative variable because it was the time of maximal response and the point where the most robust phenotypic difference between the 2 strains could be observed (Figure 1).[7] We, therefore, sought to look for specific genetic determinants of the stress response using RIS originating from reciprocal crosses of BN.Lx and SHR. In this RIS set, the strain distribution patterns of BT reached at T20 as measured under both normal and high Na intake was a continuum, indicating the polygenic nature of these phenotypes, with a high correlation between the 2 diets ($r$=0.83; $P$<0.0001; Figure 2). The progenitor strains, BN.Lx and SHR, did not stand at the extremes of distribution, which indicates that alleles increasing or decreasing the stress response could be found in both progenitor strains, which is suggestive of loci interaction. Figure 2 illustrates that the degree of genetic variance (interstrain variance) largely exceeded the intrastrain variance attributable to the environment, underlying the predominance of genetic contribution to the trait. The heritability of T20 (which includes a shared environment) was estimated at 72% and 66% under normal and high Na intake, respectively. This phenotype was selected because it has a high heritability compared with phenotypes involving initial temperature, such as change in temperature ( $\Delta$T), and initial rate of increase, for which heritability was 59% and 49%, respectively.

### Total Genome Linkage Search

The T20 phenotype was measured in 25 strains on a normal salt diet and in 24 strains on a high-salt diet. This analysis showed 2 QTLs on Chrs 10 and 12 (Figure 3) that were significantly associated with the T20 phenotype. The most significant association was found on Chr 10 (*Myh3*; $r$=0.58; $P$=0.0017; logarithm of odds [LOD] score, 2.2; Figure 3a). This effect had greater significance when the stress response was measured in rats fed a high Na diet ($r$=0.72; $P$=0.00002; LOD score, 4.0). The effect of this QTL explains the 34% and 52% of the T20 variance on normal and high Na intakes, respectively.

Another QTL was found on Chr 12, with a peak at the *Lsn2* marker ($r$=0.49; $P$=0.014; LOD, 1.5; Figure 3b). Its effect on T20 was increased by high Na intake ($r$=0.69; $P$=0.000094; LOD, 3.3). The statistical significance of these results satisfied the stringent statistical criteria proposed by Neumann[20] that are required for genome scan when using RIS.

### Impact of Chr 10 QTL on the Stress Response: RNo10 Congenic Strain

Chr 10 (RNo10) congenic rats were used to further assess the impact of the locus identified in RIS. Figure 4a shows the size of the segment of BN Chr 10 transferred onto a SHR genetic background. On both normal and high Na diets, SHR.BN-RNo10 congenic rats displayed a lower T20 than SHR (Figure 4b). In accordance with the results obtained with the genome scan in RIS, the observed difference between

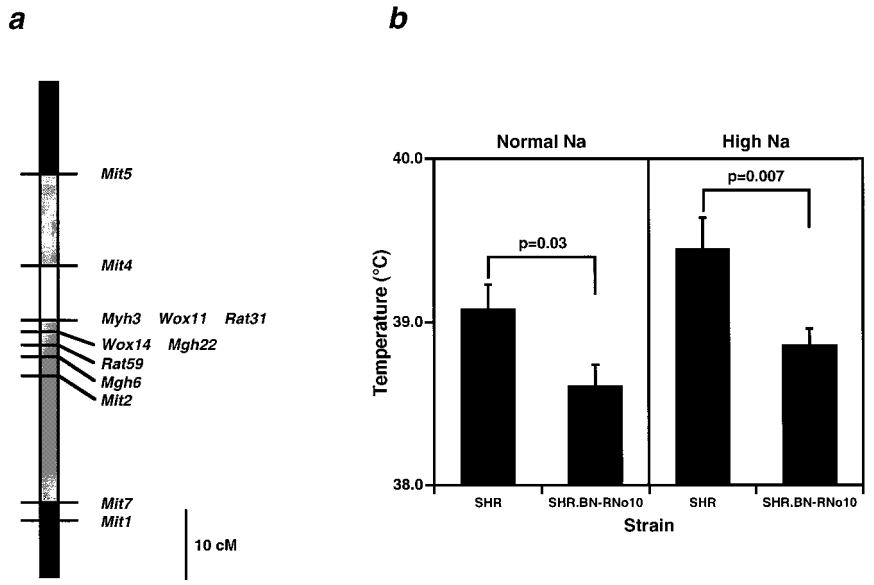


**Figure 4.** Impact of BB genotype at *Myh3* marker on stress response for SHR.BN-RNo10 congenic strain. a, Characterization of RNo10 segment of BN transferred to SHR in SHR.BN-RNo10 congenics studied. Markers *D10Mit4*, *D10Wox12*, *D10Rat31*, *D10Wox11*, and *Myh3* were homozygous to BB in all rats studied. *D10Mit7*, *D10Mit5,* and *D10Mit1* (*Ace*) were of HH genotype, and residual heterogeneity was found in *D10Wox14* (*D10Mgh22*), *D10Mgh6*, *D10Mit2,* and *D10Rat59*. White bar denotes region of BB homozygosity, whereas black bars denote background HH homozygosity. Gray bars are regions where heterozygosity was found or cannot be excluded. b, Impact of BB genotype at *Myh3* marker on thermal stress response. SHR.BN-RNo10 indicates congenic rats bearing segment of BN Chr 10 that is homozygous for BB at *Myh3* marker on SHR genetic background. $P=0.03$ and $P=0.007$ when stress is measured under normal and high Na diets, respectively, compared with SHR (*t*-test; n=6 and n=8 under normal Na and n=6 and n=9 under high Na for SHR and congenics, respectively).

SHR.BN-RNo10 and SHR had a greater significance with high Na intake (Figure 4b). Furthermore, the congenic paradigm narrowed down the region impacting on T20.

## Negatively Correlated QTLs

Negatively correlated QTLs (ie, where BN alleles are associated with a higher T20 value) were also found when the stress response was measured in rats under normal Na intake. The most suggestive one was identified on Chr 11, with a peak at the *Mit2* marker ($P=0.0008$; LOD, 2.5; $r=-0.65$). Another negative QTL for T20 on normal diet was on Chr 8 by the *Cebr92s2* marker ($P=0.0046$; LOD, 1.9; $r=-0.55$). Interestingly, a locus for hyperactivity in the WKHA rat has been localized in the same region.[21]

## Consomic Strains for the Y Chr: Modulation of the Stress Response

Because the set of RIS used in this work originates from reciprocal crosses of BN.Lx and SHR,[8,11] we could evaluate the putative effect of the Y Chr of SHR origin on the stress response. Strains fed a normal Na diet and bearing the Y Chr of the SHR progenitor (B×H strains) had a higher T20 than strains bearing the Y Chr from the BN.Lx progenitor (H×B strains) (Figure 5a; $P=0.02$). To further analyze this effect, we studied Y Chr consomic strains on the normal Na diet. The transfer of the BN Y Chr to a SHR genetic background in SHR.BN-Y consomics significantly lowered T20 compared with SHR (38.40±0.29°C versus 39.00±0.16°C, respectively; $P<0.05$; Figure 5b). Similarly, in Y Chr consomic strains originating from WKY and SHR reciprocal crosses,[22,23] T20 was significantly lower in SHR.WKY-Y strains, which bear the Y Chr from WKY and the autosomes of SHR, than in SHR (38.64±0.21°C versus 39.15±0.07°C; $P=0.014$; Figure 5b). It is noteworthy that the removal of SHR-Y and its replacement with either BN-Y or WKY-Y produces a similar decrease in T20. However, transfer of the Y Chr from SHR (SHR-Y) to a WKY genetic background (in WKY.SHR-Y) did not increase T20 compared with WKY (38.34±0.19°C versus 38.49±0.24°C; $P$=NS). Therefore, it seems that removing SHR-Y from the SHR background has more impact than adding it to a WKY background, which suggests that SHR-Y is needed, but not sufficient for, the expression of the stress response observed in SHR. The Y Chr thus seems to have a modulating effect on autosomal QTLs of the stress response.



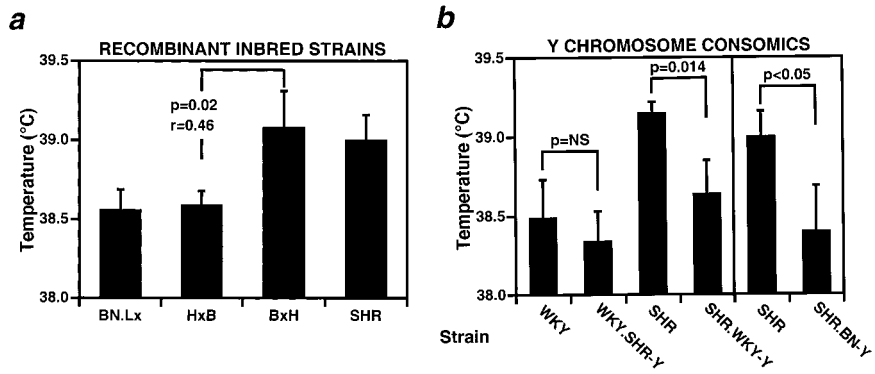


**Figure 5.** a, Impact of Y Chr on stress response in RIS. H×B indicates strains bearing Y Chr from normotensive BN.Lx progenitor (n=16 RIS). B×H indicates strains bearing Y Chr from hypertensive SHR progenitor (n=9 RIS). BN.Lx (n=7 rats) and SHR (n=5 rats) strains are included for comparison. b, Impact of Y Chr on stress response in WKY.SHR-Y and SHR.WKY-Y consomic strains. $F=5.57$ and $P=0.0082$ by ANOVA.

**T20 Values Resulting From the Genotypic Combination of *Myh3* (QTL on Chr 10) and *Lsn2* (QTL on Chr 12) Loci: Interaction Between the 2 Loci**

| | | *Myh3* (Chr 10 QTL) | |
|---|---|---|---|
| | | BB | HH |
| *Lsn2* (Chr 12 QTL) | BB | 38.42±0.06 (72) | 38.59±0.1 (14) |
| | HH | 38.63±0.06 (75) | 39.41±0.06 (81)* |

Values are mean±SEM (No. of rats per genotypic combination). BB indicates homozygous for the BN.Lx allele; HH, homozygous for the SHR allele.

*$P<0.0001$ by ANOVA vs the other 3 combinations.

### Relative Contribution of Significant QTLs on T20

We evaluated the relative contribution of significant QTLs on T20 by multiple linear regression analysis. Two significant markers were chosen as predictors of T20 (*Myh3* on Chr 10 and *Lsn2* on Chr 12), as was the Y Chr, because the impact of the latter on T20 has been confirmed in consomic strains. Data from individual rats were computed to increase the power of analysis, and the effect of the diet was evaluated. Due to multicolinearity between the primary effects and their interactions, the model cannot evaluate the impact of diet as an independent variable. We could, nevertheless, estimate the impact of the high Na diet by comparing a subset of 209 rats (21 RIS) for which stress was measured on normal and high Na diets. We observed a significant overall diet effect (ANOVA, $P=0.022$), in accordance with the results shown in Figure 3, which are suggestive of a salt-sensitive component of T20 at these loci. By combining the individual data under both dietary conditions (n=242), the following regression equation was derived:

$$\text{T20}=\text{Int}38.694+(0.19\times \textit{Myh3})+(0.26\times \textit{Lsn2})+(0.14\times \textit{Myh3}\times \textit{Lsn2})+(0.13\times \text{Myh3}\times \text{YChr})$$

in which the adjusted $r^2=0.46$.

This model predicts 46% of T20 variance. The results from multiple linear regression clearly demonstrated that the effect of the significant QTLs was not only additive, but that the 3 loci also interacted: the locus on Chr 10 with the locus on Chr 12, and the locus on Chr 10 with the Y Chr. For an interaction to be present, the effect of 1 locus depends on the genotype of the other locus.[24] This is clearly illustrated in the Table: the interaction between the loci of Chrs 10 and 12 in which the highest T20 is seen only occurs when the SHR alleles at both loci are combined (ANOVA, $P<0.0001$ versus the 3 other genotypic combinations).

## Discussion

This study revealed 2 QTLs on Chrs 10 and 12 that were significantly associated with the BT response to immobilization stress in rats. Analysis of the syntenic regions for the rat Chr 10 at the *Myh3* locus reveals a homology to the human Chr 17p13.1. An attractive candidate for the thermal response to stress is *Alox12*, a 12-lipoxygenase involved in leukotriene synthesis from arachidonic acid, which has been mapped to this region.[25] Prostaglandins may play a role in the temperature response to stress in rats because antipyretics can prevent the rise in BT due to immobilization.[5] Thus, abnormal arachidonic acid metabolism could alter prostaglandin synthesis. It should also be mentioned that a BP QTL was found in F2 rats from a SHR stroke prone (SHRSP) and WKY cross on Chr 10 in a segment encompassing *Myh3*.[26] In RIS, no QTL for BP was detected at this locus when BP was measured via intra-arterial catheter under light gas anesthesia.[9] BP during immobilization stress will have to be measured by telemetry to determine if the BP QTL found in F2 is the same as the one for T20.

Human Chr 7q21-q22 is syntenic to rat Chr 12 near the *Lsn2* locus. A malignant hyperthermia susceptibility locus (*Mhs3*) has been linked to this segment of Chr 7q. Malignant hyperthermia is a pharmacogenetic disease that susceptible individuals develop on exposure to ether and related anesthetics.[27] It is noteworthy that SHR are also hypersensitive to heat and ether.[3,4,28] Genes of calcium channel subunits and Na channel subunits mapped on human Chr 7q have been proposed as candidate genes for malignant hyperthermia.[29–31] The *Hsp27* stress gene is also a good positional candidate on Chr 12, because we have shown its modulation by SHR-Y.[32] Discussion of positional candidates must, however, be tempered by the size of the estimated confidence intervals (≈30 cM) associated with these QTLs.

Finally, of great interest is the demonstration of interactions between 2 autosomal loci (Chr 10 with Chr 12 loci) and between the Y and autosomal Chrs (Chrs 10 and Y). Interactions between primary QTLs should be expected with polygenic phenotypes, such as the stress response. Hence, the use of different crosses may further help discriminate between confounding and primary QTLs, because different genetic backgrounds may reveal or hinder significant linkages.

This work opens the way for finding the genetic determinants of susceptibility to environmental stressors. Two major QTLs were detected, with a modulating impact of the Y Chr, as ascertained in congenic and consomic strains. These results provide the first step toward a full understanding of the genetic basis of the hyperresponse to environmental stress and its relevance to cardiovascular diseases.

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