Effects of chromosome 17 on features of the metabolic syndrome in the Lyon hypertensive rat

S. Gilibert, A. E. Kwitek, N. Hubner, M. Tschannen, H. J. Jacob, J. Sassard, and A. Bataillard

Département de Physiologie et Pharmacologie Clinique, Institut des Sciences Pharmaceutiques et Biologiques, Université Lyon 1, Lyon, France; Human and Molecular Genetics Center, Department of Physiology, Medical College of Wisconsin, Milwaukee, Wisconsin; and Max Delbruck Center for Molecular Medicine, Berlin-Buch, Germany

Submitted 7 November 2007; accepted in final form 9 February 2008

1094-8341/08 $8.00 Copyright © 2008 the American Physiological Society

The metabolic syndrome is characterized by a collection of associated disorders including central obesity, dyslipidemias, hypertension, insulin resistance, and a proinflammatory, prothrombotic state, causing increased mortality from cardiovascular and renal disease (2, 11, 12, 16, 24). The etiology of the metabolic syndrome is complicated, as a collection of a range of complex traits, and is thought to involve environmental and genetic interactions. Prospective twin studies, familial segregation, and intercorrelation analyses have all supported the existence of strong genetic influences on most features of hypertension and the metabolic syndrome (15, 21, 22). Unfortunately, despite the great advances of genetic and genomic technologies over the last 20 years, the majority of the genetic determinants remain to be identified.

One way to approach many problems associated with genetic research in humans is to study the genetics of complex disease with animal models (8, 20). The Lyon hypertensive (LH) rat was selectively bred for high blood pressure (BP) from an outbred Sprague-Dawley colony in 1969 (10). Concurrently, a normotensive control strain, the Lyon normoten- sive (LN) rat, was selectively bred from the same colony. Although these strains were selectively bred on the basis of high and normal basal BP, respectively, the LH rat also shows significantly increased body weight and left ventricular hypertrophy, as well as increased levels of cholesterol, phospholipids, and triglycerides (7, 27, 28) compared with the LN rat. In addition the LH rat has enhanced BP increases after salt intake, commonly found with features of the metabolic syndrome in several rat strains (LH, SS, SHR, SBH) (3, 6, 30) and human populations (26). Moreover, the LH rat exhibits an increased insulin-to-glucose ratio (although lacking spontaneous insulin resistance or glucose intolerance) (4) and elevated fibrinogen suggesting a prothrombotic state (28). Therefore, although they were selectively bred for studies of spontaneous hypertension, LH rats have many other features in common with the metabolic syndrome, suggesting common causes or mechanistic pathways. Consequently, the LH rat is a powerful genetic model for better understanding the pathological links between hypertension, some components of the metabolic syndrome, and their associated risks for cardiovascular disease.

We previously performed (1) a genetic mapping study in a large population of F2 rats derived from a cross between LH male and LN female rats. We mapped a total of 61 quantitative trait loci (QTLs) to eight different chromosomes (RNO1, 2, 3, 5, 7, 10, 13, and 17) for traits including BP, body weight, organ hypertrophy, plasma lipids, and plasma insulin and insulin-to-glucose ratio (1). Interestingly, RNO17 contained two clusters of QTLs, one linked with body weight and organ hypertrophy and another with BP, plasma lipids, and insulin measures, with susceptibility of each trait conferred by the LH allele. Therefore, it appears that the LH RNO17 plays a role in many of the features of the metabolic syndrome, including body weight, plasma triglyceride and insulin levels, and BP.

Because multiple QTLs mapped on the same chromosome, a more efficient means to dissect the QTLs on RNO17 was to substitute the whole chromosome (a consomic strain) rather than independently generating multiple congenics, providing a resource to generate congenic lines through a simple F2 intercross (5) and the ability to combine different congenic intervals to study QTL interactions. The most likely choice of strains for...
the consomic would be LH and LN rats, because they are the strains used for the genetic linkage studies. However, the high genetic similarity between the strains made marker-assisted selection of the target chromosome a challenge. Therefore, to overcome this challenge, another normotensive control strain, the Brown Norway (BN/NHsdMewi) rat, was chosen as a chromosome donor. The LH-17\(^{BN}\) rat is an LH rat in which chromosome 17 has been fully substituted by a BN chromosome 17 by successive backcrosses and marker-assisted selection (23). Our hypothesis is that introgression of another “normal” chromosome 17 onto the background of the LH rat would result in significant differences in at least some of the traits previously mapped by genetic linkage. However, because we introduced a different “normal” allele as the target chromosome (BN vs. LN), we needed to confirm that the phenotypic differences would be consistent with those of the previous genetic cross. Therefore, males from the LH and BN strains as well as the consomic, LH-17\(^{BN}\), were characterized for the same phenotypes that mapped to chromosome 17 in the genetic cross.

**METHODS**

**Generation of Consomic Rats**

The LH-17\(^{BN}\), consomic strain, in which chromosome 17 from the BN rat was introgressed onto the LH genome background, was obtained by crossing of a male LH rat with a female BN rat followed by marker-assisted selection. The male F1 hybrids obtained were backcrossed to LH females. After every backcross, DNA was extracted from tail tips and genotyped with 14 markers spanning RNO17 at a 5-cM resolution and 146 markers spanning the remaining genome at a 10-cM resolution as previously described (17). Polymorphic markers were selected with the Genome Scanner Tool available at the Rat Genome Database (RGD; http://www.rgd.mcw.edu). Male rats heterozygous for all 14 markers were selected to generate the next backcross generation. At the sixth backcross, animals heterozygous for the full length of chromosome 17 and homozygous LH for the rest of the genome were intercrossed to fix the donor chromosome 17 as BN and to obtain founders for the consomic strain. The consomic line was then maintained by brother-sister mating.

**Single Nucleotide Polymorphism Typing**

To provide a higher-density map of the genomes of LH and BN rats, we obtained genotypes for nearly 20,000 rat single nucleotide polymorphisms (SNPs) in both strains, as well as in the LH-17\(^{BN}\) rat via a newly generated Targeted Genotyping SNP chip, based on the parallel strategy. This technology allows the parallel genotyping of over 20,000 SNPs in a single DNA sample (13, 14). Briefly, each SNP assay probe anneals target genomic DNA in a way that brings the assay probe above the iliac bifurcation through a midline abdominal incision under anesthesia with halothane (2% in oxygen). Just after surgery rats received a subcutaneous injection of Ketoprofen (5 mg/kg). During the day of surgery and during the next three following days, rats received a subcutaneous injection of penicillin G (50,000 U), and were placed into individual cages for 3 wk of recovery.

After the recovery period, BP was recorded twice (R1, R2) during the first week. BP recordings lasted 22 h/day, during which time the continuous BP signal generated by the telemetry system was sampled every 2 ms by a computerized system using Labview 5.0 software (National Instruments, Austin, TX). Data were processed off-line so as to obtain beat-to-beat values of systolic BP (SBP), diastolic BP (DBP), and mean BP (MBP). The values were averaged to obtain the baseline value (R1 + 2). The following week, rats were placed in metabolic cages. BP was recorded the first day (R3) and after a 2-day habituation period (R4) while 24-h urine samples were collected to measure total proteins (pyrogallol red colorimetric method; AU 2700 biochemistry analyzer, Olympus, Rungis, France) and sodium and potassium (Flame photometer IL943, Instrumentation Laboratory, Paris, France).

The animals were returned to their cages and, at the age of 20 wk, received a moderate salt load (1% NaCl as drinking water) for a week, followed by a high-salt load (2% NaCl as drinking water) for another week, then reduced again to 1% NaCl for 10 days, and finally tap water up to the end of the study. BP was recorded at the end of the moderate salt load (R5), at the fifth and seventh days of high salt load (R6, R7), again while returning to moderate salt load (R8, R9) and finally 2 days before the rat was killed (R10). Twenty-four-hour urine collections were performed simultaneously with the 9th recording (end of the salt load) and the 10th recording (end of the washout period).

At the end of the study, 25-wk-old animals were fasted for 16 h, given a diazepam injection (5 mg/kg ip), and killed by decapitation.

**Blood and Tissue Sampling**

A blood sample was drawn on heparin at the time of death in rats fasted for 16 h to measure insulin (radioimmunoassay, DiaSorin, Antony, France) as well as glucose, total cholesterol, and triglycerides with an Olympus automated test (AU 2700 biochemistry analyzer, Olympus). The whole heart, the left ventricle with septum, and the kidneys were dissected out and weighted. Relative weights were determined by reporting organ weight-to-body weight ratio measured just before decapitation.

**Statistical Analysis**

BN and LH-17\(^{BN}\) rats were compared to LH rats with the nonparametric Mann-Whitney test. Differences were considered statistically significant at $P < 0.05$. Results are reported as means ± SE.

---

1 The online version of this article contains supplemental material.
LH-17BN. The genotyping also shows that 45 SNPs assigned to noncontiguous SNPs were heterozygous on RNO17 in the RNO17 SNPs are polymorphic between LH and BN. Two SNPs (excluding SNPs with no genotypes available for either each were heterozygous on RNO2, 10, and 13, and one mined to be heterozygous on the genome background; how-large regions of residual BN genome. Ten SNPs were deter-mined that the error lies in the genome assembly (V3.4) to evaluate the consomic genome. On the genome background that an

68,640,275 bp) are homozygous for the LH allele, indicating RNO1. Six additional contiguous SNPs (from 66,552,277 to (5.1 Mb) were LH-specific alleles, sug-

RESULTS
SNP Analysis

The LH-17BN rat is an LH rat in which chromosome 17 has been fully substituted by a BN chromosome 17 by successive backcrosses and marker-assisted selection (19, 29) using microsatellite markers spanning RNO17 as well as the back-ground. To determine that the BN chromosome introgression was complete and the genome background was homozygous for LH, we used a rat SNP chip to perform whole genome typing of the LH, BN, and LH-17BN strains (Table 1). In total 21,032 SNPs were assayed. There was a 1.6% average no-call rate and 0.26% average heterozygosity. Of the 19,867 success-fully genotyped SNPs, 11,786 were polymorphic between LH and BN. In the LH-17BN, the SNP genotype data were used to evaluate the consomic genome. On the genome background (all chromosomes but RNO17), no alleles were fixed for a BN-specific allele, suggesting that there are not likely to be large regions of residual BN genome. Ten SNPs were deter-mined to be heterozygous on the genome background; how-ever, none was contiguous on the genome. Two SNPs each were found to be heterozygous on RNO2, 10, and 13, and one each were heterozygous on RNO1, 4, 11, and 15.

The SNP map of RNO17 consists of 812 of 853 assayed SNPs (excluding SNPs with no genotypes available for either parental or the consomic). Five hundred forty-eight of the RNO17 SNPs are polymorphic between LH and BN. Two noncontiguous SNPs were heterozygous on RNO17 in the LH-17BN. The genotyping also shows that 45 SNPs assigned to the top of RNO17 (~5.1 Mb) were LH-specific alleles, sug-

likely because of a double recombination not detected by the microsatellite genotyping during development of the consomic. One additional SNP at the very bottom of RNO17 (96,744,845 bp) was also homozygous for the LH allele, suggesting that the last 500–750 kb of the chromosome may be of the LH genome. Genotyping data for all SNPs in LH, BN, and LH-17BN can be found in Supplemental Table A.

Phenotypic Characterization of LH, BN, and LH-17BN

Because all previous genetic studies in the LH rat have used the LN strain as its “control,” we characterized both parental LH and BN strains to ensure that they were phenotypically divergent for the traits we had previously mapped. Further-more, the best physiological and genetic control for the LH-17BN strain is the LH strain, because they are nearly 97% identical at the genetic level. Therefore any phenotypic diver-

gence from the LH must be due to the BN chromosome 17.

Body weight. Figure 2 shows that BN rats exhibited a significantly lower body weight than LH rats, while the LH-17BN rat was slightly but significantly leaner than LH parents. As expected, growth curves were affected by the salt load (25). However, no interstrain differences in the slopes of the growth curves were evident.

Cardiovascular parameters. Because the differences in SBP, DBP, and MBP between the three strains (BN, LH, LH-17BN) are similar, only MBP values are reported here (Fig. 3). Under baseline conditions, MBP of the LH strain is ~40 mmHg higher than that of the BN strain. The LH-17BN consomics exhibit a modest but significant decrease in MBP from that of LH rats (~4.3 mmHg, P < 0.05). Salt load, 2% NaCl in particular, resulted in a much higher MBP increase in LH rats (+22.0 ± 2.4% of baseline) than in BN rats (+5.6 ± 1.2%), thus confirming the salt sensitivity of hypertension in LH rats (7) and the lack thereof in BN rats. In LH-17BN rats, BP remained modestly but significantly lower than in the LH parental strain. However, the high-salt diet initiated a rise in pressure comparable to LH rats (+17.0 ± 2.0% and +22.0 ± 2.4% of baseline, respectively), indicating that although the substitution of LH chromosome 17 significantly affects basal BP, it does not play a large role in the salt sensitivity found in the parental LH rat. The relative left ventricle weight was significantly higher in both LH (247 ± 1 mg/100 g) and LH-17BN (252 ± 3 mg/100 g) rats than in BN rats (199 ± 4 mg/100 g), indicating that chromosome 17 alone does not significantly influence left ventricular hypertrophy.

Table 1. SNP map for LH and BN strains

<table>
<thead>
<tr>
<th>No. of SNPs</th>
<th>Average Intermarker Distance</th>
<th>Polymorphic Between LH and BN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genome</td>
<td>21,032</td>
<td>128,790</td>
</tr>
<tr>
<td>RNO17</td>
<td>853</td>
<td>113,663</td>
</tr>
</tbody>
</table>

SNP, single nucleotide polymorphism; LH, Lyon hypertensive; BN, Brown Norway; RNO, rat chromosome.
a significant difference between the LH and consomic LH-17BN rats (3.8 ± 0.2 vs. 3.5 ± 0.2 IU/mmol). Both plasma total cholesterol and triglycerides were significantly lower in fasted BN compared with LH rats. However, only plasma triglyceride levels significantly differed in LH compared with LH-17BN rats, reduced to the level determined in the BN donor strain (Fig. 4).

**DISCUSSION**

In the present work we developed a strain of LH rats in which the whole chromosome 17 was replaced by one originating from BN normotensive rats. This allowed us to demonstrate that in the LH strain chromosome 17 contains genes that influence several parameters defining the metabolic syndrome: body weight, BP, and lipid metabolism.

The choice of developing consomic rats that differ from their parents by a whole chromosome has been dictated by the fact that BP and metabolic abnormalities are under the control of several genes (23). Therefore, it is of importance to consider pleiotropic gene effects as well as potential gene-gene interactions and cis and trans gene regulation. The consomic strategy offers the ability to address each of these scenarios as QTLs are dissected from similar QTLs on other chromosomes, while the genome context surrounding the QTL remains intact. Furthermore, a single-chromosome model to which multiple traits map facilitates studies on pleiotropic effects as well as gene-gene interactions playing a role in the phenotype, because derived congenic strains can dissect the regions and evaluate what is due to single genes with multiple phenotypic effects compared with multiple nearby genes having a collective phenotype.

Our previous full-genome scan (1) of a large population of F2 hybrids originating from a cross between LH males and LN females showed that chromosome 17 contained clusters of QTLs linked to several traits defining the metabolic syndrome. Because it appears that multiple QTLs span the whole chromosome in the LH rat, we focused our interest on better defining the role that RNO17 as a whole plays in disease in the LH strain. The choice to use BN rats, although the linkage study had been carried out on F2 (LH/H11003 LN) rats, was guided by two elements. First, the genetic polymorphism that exists between LH rats and BN rats is more marked than that existing between LH rats and LN rats, allowing for clear introgression of the entire donor chromosome. Second, the genome of the BN strain has been sequenced (23a) and extensively characterized (18) for several cardiovascular traits, including BP, renal function, body weight, and cardiac function, providing a benchmark for comparison to the LH strain. However, using a different strain required that we confirm that substitution of RNO17 of the LH with that of another “control” strain (BN) could alter the phenotypes previously mapped to this chromosome. The characterization of the parental LH and BN rats reported here confirms that marked differences exist between the parental strains. LH rats have a significantly greater body weight and higher BP, both at baseline conditions and after a high-salt diet (1% and 2% in the drinking water), indicating that the BN rat is an appropriate control strain for the devel-

![Fig. 2. Time course evolution of body weight in LH, LH-17BN, and BN rats. **P < 0.01 vs. LH.](image)

![Fig. 3. Time course of mean blood pressure (MBP) recorded by radiotelemetry in LH, LH-17BN, and BN rats. Each MBP value corresponds to the average of a 22-h beat-to-beat MBP recording. The values of the first 2 recordings (R1, R2) were averaged to obtain the baseline value (R1 + R2). *P < 0.05 vs. LH; **P < 0.01 vs. LH.](image)

![Fig. 4. Plasma total cholesterol and triglyceride levels in fasted 25 wk-old LH, LH-17BN, and BN rats. Numbers in histogram bars indicate the number of rats. **P < 0.01 vs. LH.](image)
opment of the consomic strains. Furthermore, the LH rat has a significantly elevated urinary excretion of proteins (data not shown), an increased insulin-to-glucose ratio, and higher plasma concentrations of cholesterol and triglycerides compared with the BN rat.

The introgression of a BN chromosome 17 in the LH genetic background also showed significant differences from the parental LH rats. First, the LH-17BN rat has a significant decrease in body weight and MBP compared with LH rats. The introgression of a BN chromosome 17 displayed a nonsignificant decrease in insulin-to-glucose ratio and plasma cholesterol but, interestingly, reduced plasma triglycerides to reach the level observed in BN parents. These data lead to the conclusion that replacement of a single chromosome in the LH rat is sufficient to affect some, but not all, traits related to the metabolic syndrome previously identified in the LH × LN intercross. In some cases the overall effect was reduced to the level of the BN donor strain (e.g., triglycerides), indicating that a gene(s) on this chromosome conveys a strong independent effect on the overall trait. However, in other cases (body weight, BP) the phenotype was only partially rescued, likely because of independent or interacting effects of QTLs on other chromosomes (body weight on RNO1, BP on RNO2 and RNO13). Furthermore, the nonsignificant decrease in the cholesterol and insulin measures could also be due to allelic effects of the introgressed BN RNO17 and/or the requirement of additional factors in the genome background of the F2 intercross.

While successes in mapping QTLs provide valuable genomic information, there remains a need for improving tools to be used in identifying genes that affect complex traits. We opted to generate a consomic strain because 1) multiple QTLs were present that spanned the whole chromosome and 2) consomics offer a rapid means of producing overlapping congenic strains via an F2 intercross (5). In our experience, linkage mapping generally results in defining a QTL interval to ~30 cM; by using other resources such as congenics, QTLs may be refined to intervals of ~2–5 cM. This strategy, while very powerful, still requires significant breeding and phenotyping efforts to further narrow the regions of interest. One way we might be able to further narrow the critical region in congenic strains is to study SNP haplotype structures. The SNP genotypes for LH, BN, and over 200 additional rat strains are currently being determined and released in the public domain, allowing for the determination of the haplotype structures on chromosome 17 between the strains with higher resolution than available with the previous genetic map. These data could be used to guide the generation of overlapping congenics for validation of a QTL.

ACKNOWLEDGMENTS

We thank Dr. R. Cohen for insulin measurement.

GRANTS

This work was funded in part by National Heart, Lung, and Blood Institute Grant HL-54508 (A. E. Kwitek, H. J. Jacob), N. Hubner is funded through STAR, a project supported by the 6th Framework Program of the European Union. S. Gilibert is a recipient of a fellowship from the French Society of Hypertension.

REFERENCES