Further chromosomal mapping of a blood pressure QTL in Dahl rats on chromosome 2 using congenic strains

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Received 3 January 2001; accepted in final form 3 April 2001

Dutil, Julie, and Alan Y. Deng. Further chromosomal mapping of a blood pressure QTL in Dahl rats on chromosome 2 using congenic strains. Physiol Genomics 6: 3–9, 2001.—Both linkage and use of congenic strains have shown that a region on rat chromosome 2 (Chr 2) of Dahl salt-sensitive rats (S) contained a quantitative trait locus (QTL) for blood pressure (BP). A congenic strain was made by replacing a segment of the S rat by the homologous region of the Milan normotensive (MNS) rat. Since the region was roughly 80 cM in size, a further reduction is required toward the final identification of the QTL. Currently, three congenic substrains were made by replacing smaller sections within the 80 cM. Each strain contains a specific region of MNS in the S genetic background. Two of the three congenic strains shared a segment in common, and both showed a BP-lowering effect. One of the three congenic strains carried a unique segment and had the same BP as S. Deducing the fragment shared in the two substrains having an effect, the BP QTL has to be present in a region of roughly 15 cM. In contrast to BP, heart rates of all the congenic rats were the same as that of the S rat. Thus BP and the heart rate are under the control of independent genetic determinants.

The mapping of quantitative trait loci (QTL) for blood pressure (BP) using animal models has been greatly facilitated by the genetic approach, such as linkage analysis and the use of congenic strains (1, 7, 13). In our previous work, regions on rat chromosome 2 (Chr 2) were shown to contain a QTL first by linkage analysis (2, 3, 5). The evidence suggested (2) that there might be two QTL separated by more than 40 cM, one near the locus for the Na+-K+-ATPase α-subunit gene (Atp1a1) and another near the locus for the angiotensin receptor AT1B gene (Agtr1b) in an F2(S × MNS) population. The presence of a QTL was proved by the use of two congenic strains (3). In one congenic strain, the chromosome region of interest from the Dahl salt-sensitive strain (S) was replaced by that of the Milan normotensive (MNS) rat (3). The length of the region involved in making the congenic strain was ~80 cM.

To further narrow down the region containing the QTL, we made congenic substrains from the original strain, S.MNS-D2Mit6/Alcohol dehydrogenase (Adh) (3), and studied BPs of these strains using telemetry.

METHODS

Animals. The S rat used for making congenic strains was provided by Dr. John Rapp. The original litter of five male and five female S rats was obtained directly from Dr. John Rapp. From these rats, the first independent breeding of them by Alan Deng was done on February 6, 1998, followed by three more generations at the Medical College of Ohio, Toledo, OH. On December 7, 1998, two litters of S rats were shipped to our current location at Montreal, Canada. After a period of quarantine and at the third generation (born on December 10, 1999) bred from the shipped S rats, the current telemetry studies began. Therefore, a total of six generations had elapsed from the time the rats originated from Dr. John Rapp to our first telemetry study. The genomic DNA for every S rat used for maintaining the strain and BP studies at each generation has been extracted and verified by genotyping.

To ensure that the S strain remains as genuine as the SS/Jr rat directly obtained from Dr. J. Rapp in our facility, a rigorous and strict quality control procedure has been instituted. It consists of two parts: 1) genetic testing and 2) physical distinction. In an approximate genomic scan, 88 markers roughly evenly spaced (on average 10–15 cM) throughout the rat genome were tested at every third generation of breeding starting from generation 5. At every generation, at least one marker randomly chosen for each chromosome was genotyped, and at every other generation, at least two to three markers for each chromosome were genotyped. Each DNA sample for every S rat tested was compared with the true S DNA standard that we used in our original work (3). At the time the current work was done, there were only S, S.M, and S.M strain derivatives present in our animal facilities (see below). If any potential genetic contamination of the S strain would happen, then it could only come from an accidental breeding between the S and S.M strains. This possibility was categorically eliminated by genotyping markers (shown in Fig. 1) on the rats of the S strain in each generation. Moreover, all our S rats have, in addition to a skin tag, an ear mark punch hole designed to facilitate their identification and to reduce potential handling errors.

The original congenic strain used to initiate the study is designated as S.MNS-D2Mit6/Adh and is the same as published previously (3). This original congenic strain is abbreviated as S.M. In brief, it was made by eight consecutive
backcrosses, then consequently putting the MNS chromosome region between D2Mit4 and Adh markers on the S genetic background. This strain, therefore, is homozygous MNS (i.e., MM) for the region in question and homozygous SS for the rest of the genome. This conclusion has been supported by the genotyping of 57 markers scattered throughout the rat genome other than on Chr 2 (data not shown). Figure 1 contains the Chr 2 map and the chromosome fragment in question in the congenic strain.

The S.M strain was transferred to Alan Deng from Dr. John Rapp (January 14, 1998) and has been maintained for approximately the same generations as S. Two S.M litters were then shipped along with the S rats to Montreal at the same time as S. The authenticity of the strain has been

Fig. 1. Chromosome 2 (Chr 2) regions replaced in the original congenic strain and substrains and the quantitative trait locus (QTL) region mapped. The linkage map is essentially the same as published previously (4), which is based on an F2(S × MNS) population. Numbers to the left of the linkage map are units in cM. The order of most of the loci on the map has been initially determined by linkage using the MAPMAKER program, then verified by scoring crossovers during construction of congenic substrains. RH map refers to the map based on rat/hamster radiation hybrids, in which units are in centirays (cR). S.M represent the original congenic strains S.MNS-Adh/D2Mit6 (3). S.M2, S.M5, and S.M6 are substrains that have been derived from S.M. Solid bars under congenic strain and the substrains symbolize the S chromosome fragments that have been replaced by that of the MNS rat. The entire region indicated by solid bars and junctions between the solid and open bars are homozygous MM on the map for all the markers listed in the corresponding positions. Open bars on ends of solid bars indicate the ambiguities of crossover breakpoints between markers. Junctions between solid and open bars as well as ends of chromosome regions of interest in each strain are connected by dotted lines to the marker positions on the map. Adh, alcohol dehydrogenase; Agtr1b, angiotensin receptor type 1B; Atp1a1, Na+/K+-ATPase α1-subunit; Camk2d, calmodulin-dependent protein kinase II-δ; Cpb, carboxypeptidase B; Fgg, fibrinogen-γ; Gca, guanylyl cyclase A/atrial natriuretic peptide receptor; Hsd3b, 3-hydroxysteroid dehydrogenase/17β-isomerase; Nep, neutral endopeptidase; Prlr, prolactin receptor. The rest of the markers are anonymous (http://waldo.wi.mit.edu/rat/public/). S.M2, S.M5, and S.M6 are S.MNS-D2Mit6/D2Rat303, S.MNS-Nep/D2Mit14, and S.MNS-Nep/Gca, respectively. S, Dahl salt-sensitive strain; MNS, Milan normotensive strain.

Physiol Genomics • VOL 6 • www.physiolgenomics.org
established by genotyping the markers between D2Mit6 and Adh on Chr 2 (Fig. 1).

Protocols for handling as well as maintaining animals were approved by our institutional animal committee. All the procedures for the experiment were in accordance with the guidelines of local, provincial, and federal regulations.

Breeding scheme for generating substrains. Rats of the original congenic strain, S.M (Fig. 1) were first bred with S to produce F1 rats, which in turn were intercrossed to produce F2. An F2 rat with crossovers in the region between D2Mit6 and Adh markers was retained, then backcrossed to an S rat to duplicate the region of interest. A female and a male backcrossed rats were crossed to finally generate rats homozygous MM for the region of interest, but homozygous SS for the rest of Chr 2 and rest of the genome. The progeny of each of these crosses constitute a congenic strain subdividing the initial segment involved in S.MNS-D2Mit6/Adh. S.MNS-D2Mit6/D2Rat303 is simplified as S.M2; S.MNS-neutral endopeptidase (Nep)/D2Mit14 as S.M5; and S.MNS-Nep/atrial natriuretic peptide receptor/guanylyl cyclase A (Gca) as S.M6. The chromosome region homozygous MM in each substrain is shown as solid bars in Fig. 1.

The first backcross between the S and S.M rats occurred on February 6, 1998, and all the substrains were finally fixed to the MNS homozygosity for each region in question by January 9, 2000. The chromosome region homozygous MM in the substrain and the original S.M strain are shown as solid bars in Fig. 1. All the markers in the region were genotyped for each congenic strain in question.

Preparation of rats for BP measurements. The mating pairs of the S and congenic strains to be studied were bred simultaneously and in the same facility. Male rats were chosen from two separate litters of the same strain when possible, to minimize potential litter effects. The chosen rats were weaned at 21 days of age, maintained on a low-salt diet (0.2% NaCl, Harlan Teklad 7034), and then fed a high-salt diet (2% NaCl, Harlan Teklad 94217) starting from 35 days of age to the end of the experiment.

Telemetry probes of an implantable device (model PA-D70) were implanted when rats were 56 days old with their body weights between 250 and 320 g. The procedure follows that of Data Sciences (St. Paul, MN). Before the surgery, the rats were anesthetized by the inhalation of isoflurane at a dose of 1.5–2%. The pressure catheter, which contains the biocompatible gel at the tip and the noncompressable fluid connecting the tip to the pressure sensor, was implanted through the femoral artery until it reached all the way up to the abdominal aorta. It is secured by sutures to the blood vessel. The telemetry device body, which includes the pressure sensor, the reusable electronics module, and a battery, was fixed to the muscle wall and was left in the abdominal cavity. After the surgery, the rats were allowed to recuperate for 10–17 days. In the first 3 days of recuperation, the rats were fed Jell-O containing the analgesic buprenorphine at 0.5% mg/kg. Afterward, they were given a diet of Ensure milkshake with chocolate in addition to the 2% NaCl regular food. This supplement usually lasted up to 5 days to facilitate their appetite and thus weight gain. In total, the rats are allowed at least 10 days for the postoperative recovery.

BP measurement. The telemetry system from Data Sciences was used. Each telemetry probe was calibrated before and cleaned after each usage according to the manufacturer’s instructions. A dedicated technician was thoroughly trained by Data Sciences for the procedure of telemetry. Statistical analysis. Repeated measures ANOVA followed by Tukey in the SYSTAT 9 program (SPSS Sciences, Chicago, IL) was used to compare the significance level for a difference or a lack of it in BP between a congenic strain (or substrain) and the S strain. In the analysis, BP was compared at each day for the period of measurement among the strains.

DNA extraction and genotyping. DNA for each rat was extracted by tail biopsy using a Qiagen genome kit, and the genotype of each rat was determined by PCR on the methods previously published (2–4).

Radiation hybrid mapping. A rat/hamster (RH) panel of 96 radiation hybrids was purchased from Research Genetics (Huntsville, AL; http://www.resgen.com/). For chromosome mapping, each marker was genotyped using RH by PCR according to a previously published protocol (2–4). To locate a marker of interest onto an existing RH framework map, the results of genotyping were entered into a web site, http://rgd.mcw.edu/RHMAPSERVER (9).

RESULTS

BP study designs. The basic design of raising animals is similar to our previous congenic work in terms of age and sex and for the timetable of dietary treatments (3). The most apparent difference is that BP measurements in the current study were direct and continuous for a period of 10–20 days. All the BP components were measured, including mean (MAP), systolic (SAP), and diastolic (DAP) arterial pressures.

BP measurements. Readings for each BP parameter were recorded every 2 min during a 24-h period for the duration of the experiments. As a result, 60/2 × 24 = 720 measurements were collected daily. For the simplicity of presentation, each point in the graphs in Fig. 1 represents averaged 24-h readings taken from every 4 h. Each error bar represents SE for all the individual data points collected from every 4 h for all the rats of that strain. MAPs, SAPs, and DAPs for all the strains are given to show their magnitudes and ranges.

Mapping of BP QTL by analyzing BP effects associated with chromosome segments. Figure 2 shows the comparison of MAPs, SAPs, and DAPs of S rats with those of congenic rats. The chromosome regions containing MNS substitutions in each congenic strain are shown in Fig. 1. A total of nine S rats were pooled from separate and independent cohorts of BP measurement during different periods of time, because the differences in S rats among the separate cohorts turned out not to be statistically significant (P > 0.39). The BP data from two independent cohorts for the S.M2 substrain were pools also for the same reason (P > 0.87).

As shown in Fig. 2, MAPs, DAPs, and SAPs were significantly lowered (P < 0.001) in the S.M (n = 5), S.M5 (n = 4), and S.M6 (n = 7) congenic strains compared with that of the S (n = 9) strain. But the difference in MAP, DAP, and SAP between the S.M2 (n = 8) and S (n = 9) strains is not significant (P > 0.86).

Heart rates of the rat strains were measured and are shown in Fig. 3. Each graph represents the rat strains compared simultaneously during the same period of time. The heart rates of S rats were significantly different from one cohort to another and thus could not be
pooled. This fluctuation probably reflects mostly the effects of the environment.

**DISCUSSION**

BP of the original congenic strain S.M was shown to be significantly lower than that of the S strain (Fig. 2), thus confirming the presence of BP QTL in the chromosome segment involved. To narrow down the location of the QTL, the original segment was split into subsections (shown as solid bars for S.M2, S.M5, and S.M6 in Fig. 1).

Our congenic strains differ from the S strain essentially in the Chr 2 regions in question, i.e., homozygous MM (Fig. 1) and homozygous SS for the rest of the genome as shown by genotyping a number of markers across the rat genome (data not shown). More importantly, no matter what effects the remaining MNS genome had in the S background in the congenic S.M strain, it definitely did not influence BP, because S.M2, which was entirely derived from S.M, showed the same BP as that of S. This fact indicates, without doubt, that the BP-lowering effect shown in S.M is entirely due to
the Chr 2 segment not shared between S.M and S.M2. Therefore, at least one BP QTL has to exist in the Chr 2 segment between D2Mit9 and Adh markers.

A region containing a BP QTL can be narrowed further by examining S.M5 and S.M6. By comparing these substrains, one can reasonably conclude that a region containing a BP QTL should be located in a minimum segment shared by both substrains, i.e., between D2Rat166 and D2Mgh10 (roughly 15 cM). In this case, the same region was tested independently two times and turned out to have a BP-lowering effect every time.

In contrast to BP, heart rates of the all the congenic strains are the same as that of the S rat ($P > 0.49$) when averaged at every 24 h. This is mostly striking when noticing that MAPs of S.M5 and S are significantly different and MAPs of S.M2 and S are not (Fig. 2D), whereas the heart rates of all three strains were not significantly different (Fig. 3C). These observations indicate that a QTL present in the chromosome region

Fig. 2—Continued
of interest influences only BP, not heart rate. Thus BP and heart rate are controlled independently by different genes.

Upon closer examinations, it appears that there could be a sizable difference in heart rates between S and both S.M (Fig. 3B) and S.M6 (Fig. 3F) within an 24-h period. It is to be determined, however, whether these differences within 24 h could be genetic, because S.M5 had a similar heart rate as S (Fig. 3D), whereas S.M6 had a slower heart rate (Fig. 3F). Both S.M5 and S.M6 shared a chromosome segment in common (Fig. 1). They are reports of genetic determinants for heart rates independent of BP in both humans (16) and the spontaneously hypertensive rats (11).

Although S.M5 contained Atp1a1, whereas S.M6 did not, both showed a BP effect with a similar magnitude (Fig. 2, B and C). This fact would argue against the candidacy of Atp1a1. However, since the possibility of an epistatic gene-gene interaction among potential multiple QTL in the same region could not be ruled out at this point, Atp1a1 could not be excluded as a candidate QTL. A definitive proof will come from a congenic strain involving a minimum chromosome segment containing the Atp1a1 locus.
For a more in-depth study of a BP QTL, especially when one attempts to study a time course relationship, telemetry appears to be more appropriate, because it can provide continuous measures for all components of BP. Moreover, if DAP and SAP are controlled differently, then they might contain separate genetic determinants also. Telemetry is ideal for monitoring each separate component of BP and following it with time. On the other hand, the BP measurements obtained by telemetry have an environmental element not present in tail cuff, that is, the stress induced by surgery. It is reassuring, though, that both tail cuff (3) and telemetry (the present study) showed a BP-lowering effect for S.M.

The work of investigators using other hypertensive models has also shown that there is a QTL in the same broad chromosome region either in linkage analysis (12, 14, 15, 17) or using congenic strains (10). The QTL of interest appears to be common to most of the hypertensive rat strains. By extrapolation, the QTL might be conserved in other mammals potentially including humans. Although our experiments put rats on 2% NaCl, this setup was largely designed to hasten the expression of hypertension in a shorter period of time than otherwise would have been without it. The salt-sensitive nature of the S rats may restrict the general applicability of other BP QTL discovered from the S strain to different hypertensive strains.

Because the S.M2 substrain does not show a BP effect, the Agtr1b gene cannot be supported as a candidate for a BP QTL. Combining our previous sequencing analysis (6), the Agtr1b gene does not appear to be a candidate. The various results of linkage analyses conducted in different crosses (2, 6, 8) most likely reflected statistical artifacts. Once again, our current work illustrates the importance of the congenic approach to either support or refute the results of linkage analyses.

The region containing the BP QTL in the rat shares a conserved synteny relationship with a segment of mouse Chr 3 and segments of human Chr 1 and 4 (http://www.well.ox.ac.uk/rat_mapping_resources/Comparative_maps/compa_map_chr02.html). A fine mapping of the QTL in question may further refine the exact portion of the human chromosome with which it has a conserved synteny and eventually help unravel some of the genetic determinants in human hypertension. As the human and mouse genome projects progress, genomic information obtained might also contribute to the identification of the QTL in the rat.

We thank Dr. J. Rapp for providing the S strain and for collaborating on making the original congenic strain, S.MNS-Adh/D2Mit6. We also thank Dr. J. Tremblay in helping set up the telemetry system and Marie-Claude Guertin in statistical analyses.

This work was supported by American Heart Association National Center Grant 0140149N and Canadian Institutes for Health Research Grant MOP36378 to A. Y. Deng. A. Y. Deng is an Established Investigator of the American Heart Association.

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