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Linkage map and congenic strains to localize blood pressure QTL on rat Chromosome 10

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Abstract. Our purposes were to develop a linkage map for rat Chromosome (Chr) 10, using chromosome-sorted DNA, and to construct congenic strains to localize blood pressure quantitative trait loci (QTL) on Chr 10 with the map. The linkage mapping panel consisted of three F_2 populations totaling 418 rats. Thirty-two new and 29 known microsatellite markers were placed on the map, which spanned 88.9 centiMorgans (cM). The average distance between markers was 1.46 cM. No markers were separated by more than 6.8 cM. Four congenic strains were constructed by introgressing various segments of Chr 10 from the Milan normotensive strain (MNS) onto the background of the Dahl salt-sensitive (S) strain. A blood pressure QTL with a strong effect on blood pressure (35–42 mm Hg) when expressed on the S background was localized to a 31-cM region between *D10Mco6* and *D10Mco1*. The region does not include the locus for inducible nitric oxide synthase (*Nos2*), which had been considered to be a candidate locus for the QTL.

Introduction

Genetic markers on rat Chr 10 cosegregate with blood pressure in certain crosses involving both Dahl salt-sensitive (S) and spontaneously hypertensive rats (Hilbert et al. 1991; Jacob et al. 1991; Deng and Rapp 1992, 1995). In an F_2 population derived from S and the Milan normotensive strain (MNS), we previously found (Deng and Rapp 1995) that a very broad region of Chr 10 linked to blood pressure, suggesting the possibility of two adjacent blood pressure quantitative trait loci (QTL).

The purposes of the present work were to: a) develop new microsatellite markers for rat Chr 10 with chromosome-sorted DNA; b) construct an improved linkage map of Chr 10 with emphasis on generating markers polymorphic between S and MNS rats; and c) construct congenic strains for various segments of Chr 10 with the improved map in order to map the blood pressure QTL(s) more accurately.

Materials and methods

Animal procedures. Inbred Dahl salt-sensitive (SS/Jr) and inbred Dahl salt-resistant (SR/Jr) rat strains (Rapp and Dene 1985) were from our colony at the Medical College of Ohio and will be referred to hereafter as S and R rats. Lewis rats (LEW/NCrlBR) were obtained from Charles River Laboratories (Wilmington, Mass.) and are referred to as LEW. Wistar-Kyoto (WKY/NHsd) and Brown Norway (BN/SsNHsd) rats were obtained from Harlan Sprague-Dawley (Indianapolis, Ind.) and will be referred to as WKY and BN, respectively. The Milan normotensive strain (MNS) was obtained from the Genetic Resource Section of the National Institutes of Health (Bethesda, Md.). Three large F_2 populations of male rats that were previously used in various linkage studies (Rapp et al. 1994; Deng and Rapp 1992) were used here as a mapping panel: F_2 (S × MNS), n = 171; F_2(S × LEW), n = 151; and F_2(S × BN), n = 96.

Congenic strains were constructed by placing various regions of rat Chr 10 from MNS rats on the genetic background of the salt-sensitive S rat by a standard breeding protocol (Rapp and Deng 1995; Silver 1995). S and MNS were crossed to produce F_1, and F_1 were backcrossed to S to produce the first backcross population. Such backcrosses were repeated seven more times, always selecting as breeders for the next generation those rats that were heterozygous for genetic markers defining the segment to be transferred. After a total of eight backcrosses, heterozygotes were mated to obtain rats homozygous for the MNS alleles throughout the Chr 10 region of interest. Once the region was fixed, congenic strains were maintained by brother–sister mating.

Blood pressure of congenic rats containing substituted regions of Chr 10 was compared with blood pressure of the recipient S strain. Strains to be compared were bred at the same time, and litters were culled to 10 pups/litter. Pups were weaned at 30 days of age and identified with a numbered skin clip placed in the back of the neck (National Band and Tag Co., Newport, KY). They were housed four cage (two S and two congenic, or one S and three congenic), and a diet containing 2% NaCl, Teklad diet 94217 (Harlan Teklad, Madison, Wis.) was fed starting at 40 days of age; rats had free access to water. After 24 days on the diet, blood pressure was taken at daily sessions for 4 days by the tail-cuff method (Bunag and Butterfield 1982) in the conscious, restrained rat with equipment made by IITC Inc. (Woodland Hills, Calif.). Rats were warmed to 28°C for blood pressure determination. At least three consistent blood pressure readings at a given daily session were obtained and averaged as that session's reading. The four blood pressure sessions for each rat were performed by two different operators, who did not know the identity of the rats; each operator performed two sessions on each rat. The averages of all four sessions' readings were averaged as the blood pressure of the rat. Rats were killed by an overdose of pentobarbital, and body weight and heart weight were obtained.

Generation of markers from Chr 10-sorted DNA. Rat Chr 10 was obtained by bivariate flow cytometry as described by Hoebee and associates (1994). The DNA was amplified with a degenerate oligonucleotide-primer (6-MW primer: 5′CCGACTCGAGNNNNNNATGTGG-3′ which contains a restriction site for *Xho*1 near the 5′ end) as described by Telenius and colleagues (1992). Stock chromosome 10-sorted DNA was reamplified with the 6-MW primer to provide material for library preparation. PCR-amplified DNA was then digested with *Xho*1 and was cloned into the *Sal*1 site of the vector pT7T3 U18 (Pharmacia Biotech, Piscataway, N.J.). Clones were screened with a mixture of ^{32}P end-labeled $(CA)_{15}$ and $(CT)_{15}$ oligonucleotides by standard protocols for colony hybridization (Sambrook et al. 1989). Plasmid DNA from positive clones was isolated with Qiagen Plasmid Mini Kits (Qiagen, Inc., Chatsworth, Calif.), and double-stranded DNA was sequenced by cycle sequencing with a kit from Epicentre Technologies (Madison, Wis.). Samples were run on an LI-COR (Lincoln, NE) model 4000L automated DNA sequencer. PCR primers were designed around microsatellites with PrimerSelect (DNASTAR Inc., Madison, Wis.). PCR conditions for each primer pair were optimized in buffer containing 1.5 mM $MgCl_2$ for annealing temperatures between 45° and 65°C.

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Generation of a new polymorphic marker for the rat Ngfr gene. Because *Ngfr* markers available in the literature (Serikawa et al. 1992; Deng and Rapp 1995) are not polymorphic between the S and MNS rats, a new *Ngfr* marker was generated. *Ngfr* sequence data (accession number X05137) were used to design PCR primers: forward 5′CTTAATGTCAGCCCTCG3′ and reverse 5′ATGAAGCTCAGCTCTGC3′. The primers were then used to amplify rat genomic DNA to generate a probe of 215 bp. This probe was used to screen a rat λ-phage genomic library. Four clones were obtained. A 3-kb *Bam*HI fragment shared by the clones was found to contain a rare $(CCTGCTG)_{20}$ repeated region. PCR primers were designed around this repeat and are given in Table 1; the marker is designated *D10Mco31*.

Generation of markers using primer walking. The Promotor Finder DNA Walking Kit for the Rat (Clontech Laboratories, Palo Alto, Calif.) was used to find polymorphic markers adjacent to known markers that were not informative between S and MNS. This kit makes use of the PCR-suppression effect (Siebert et al. 1995; Lukyanov et al. 1994) combined with vectorette PCR features (Lagerström et al. 1991). A special adaptor is ligated to the ends of DNA fragments generated by separate digestion of rat genomic DNA with five restriction enzymes (*Eco*RV, *Sca*I, *Dra*I, *Pvu*II, *Ssp*I). These separate DNA digests ("libraries") are used as templates for PCR with the adaptor primer and sequence specific primer followed by a second PCR with nested adaptor primer and nested sequence specific primer. Sequence information around microsatellites of known map position was used to design the sequence-specific primers for walking upstream or downstream from the microsatellites. These primers were 27–33 nucleotides in length with a G/C content of 40–60%. PCR was performed with Advantage *Tth* Polymerase Mix (Clontech Laboratories, Inc.) and the cycling parameters described in the walking kit. The sizes of the PCR products were estimated by electrophoresis through 1.2% agarose gel. The presence of a microsatellite in the newly amplified DNA was determined by Southern blot-hybridization with a mixture of ^{32}P end-labeled $(CA)_{15}$ and $(CT)_{15}$ oligonucleotides. PCR products containing new microsatellites were cloned with the TA Cloning Kit (Invitrogen, San Diego, Calif.) and then sequenced. Flanking sequences around the new microsatellites were used to design primers. When no new microsatellites were found in the first round of primer walking, a second round was performed.

Genotyping. DNA for genotyping F_2 populations was extracted from frozen livers by the method of Blin and Stafford (1976). DNA for genotyping of rats for development of congenic strains was obtained from tail biopsy tissue extracted with the QIAamp Tissue Kit (Qiagen). Genotyping was done as previously described (Deng and Rapp 1992) with PCR amplification of microsatellite markers resolved by gel electrophoresis in agarose or polyacrylamide. PCR amplification was performed as follows with an MJ Thermocycler (M.J. Research, Watertown, Mass.): 95°C for 5 min and continued for 35 cycles of 94°C for 40 s, 45–65°C (depending on the primer) for 40 s, 72°C for 1 min 30 s plus extension 1 s/cycle, followed by an additional 72°C for 5 min. In three cases, the size differences between strains were too small to resolve with the relatively large PCR products obtained, but these differences could be resolved after reducing the PCR product size by restriction digestion.

Linkage and statistical analysis. Linkage maps and QTL localization were done with the MAPMAKER/EXP (Lander et al. 1987; Lincoln et al. 1992b) and MAPMAKER/QTL Programs (Paterson et al. 1988; Lincoln et al. 1992a) obtained from Eric Lander (Whitehead Institute, Cambridge, Mass.). The MAPMAKER programs also detect potential genotyping errors based on results of flanking markers. These samples were always retyped to confirm or correct the results. A one-way analysis of variance (ANOVA) or *t*-tests for comparing blood pressures among congenic strains was done with SPSS programs (SPSS, Chicago, Ill.).

Results

Screening the Chr 10-specific library for microsatellites. After screening approximately 30,000 clones for simple sequence repeats, we obtained 121 positive clones. These clones were analyzed by restriction digestion with *Xba*I and *Hin*dIII, and compared with one another by PCR and/or sequencing. Eighty-one of these clones proved to be unique. Their inserts ranged in size from 100 to 1300 bp. The majority (more than 80%) of analyzed clones contained $(CA)_{6-54}$ repeats. About 12% of clones contained $(CT)_{12-27}$ repeats. Approximately 8% of clones contained complex microsatellites composed of $(CA)_n(TA)_m$ or $(CA)_n(CT)_m$ repeats. Less than 10% of the clones contained more than one microsatellite.

From the 81 unique clones containing microsatellites, 59 were used for generation of primers for further analyses. The other clones could not be used because the microsatellite was either located too close to the end of the insert, or we could not design appropriate primers owing to the nature of the sequence around the microsatellite. With genomic DNA as the target, PCR products of expected size were obtained for 49 clones. Another ten clones failed to amplify a product of the expected size under the conditions used. BLAST searches (Altschul et al. 1990) of GenBank were run with the sequences of the 59 clones from which primers were designed. Only one of these clones displayed a noteworthy similarity to any previously reported sequence. *D10Mco17* has a 95% homology to a human STS (accession #F00344, #N21525, #R20767), which in turn is highly homologous to a portion of the three prime untranslated region of a mouse cofilin isoform cDNA (accession #L29468). BLAST search also revealed that 13 of these 59 clones (22%) contained LINE, B2, and ID repeats. In two of these cases, it was possible to design primers avoiding these repeats. However, in 11 cases, at least one primer overlapped the repeat region. Three of these clones gave good products, while PCR products from another two clones had a high background. Six of these 11 clones failed to yield the expected PCR products. This represents 60% (6/10) of all failures, a result consistent with previously reported observations for bovine libraries (Stone et al. 1995).

Size polymorphisms between S and R, LEW, MNS, WKY, BN were determined for 35 microsatellites. Fourteen microsatellites were not polymorphic in these strains. Thirty-four microsatellites were useful for genotyping at least one of the following populations: F_2(S × LEW), F_2(S × MNS) and F_2(S × BN). Linkage analysis revealed that 6 out of 34 markers did not link to Chr 10. One of them (*D5Mco1*) linked to Chr 5, another (*D12Mco1*) to Chr 12, and four of them (*DOMco18, 19, 20,* and *21*) have not yet been linked to any chromosome. The remaining markers were mapped to rat Chr 10 as expected (Table 1).

Genotyping of available markers from the rat Chr 10. Chromosome 10 markers used previously by us are given in Deng and Rapp (1995). In order to obtain additional markers for F_2(S × MNS) and F_2(S × LEW) populations, markers already assigned to Chr 10 by others (Jacob et al. 1995) were tested for polymorphism between S and MNS and S and LEW strains (see Table 1 for references). Fourteen informative markers were found for these crosses (Table 1) and were genotyped.

Obtaining additional markers in the area between Ace and Nos2. Primer walking was performed from the sequence surrounding *D10Mco12*, which is not polymorphic between either S and MNS or S and LEW, but is polymorphic between S and BN. A $(TG)_{19}$ microsatellite (*D10Mco30*, Table 1) was found. It was polymorphic between S and MNS and between S and LEW, and was mapped to the expected region. Primer walking was also performed in both directions from the *D10Mgh5* marker. A $(GT)_{17}$ microsatellite (*D10Mco29*) was found within 1 kb of *D10Mgh5* in one direction, and a $(GT)_7$ (*D10Mco32*) was found about 2 kb in the other direction, but neither was polymorphic in any of the populations used for mapping.

Twenty-six markers from a region of mouse Chr 11 between

Table 1. Genetic markers for rat Chromosome 10.

Name	GenBank No	Primer sequences or sources	Product size	Strain polymorphisms
DOMco5	U5390	AGAATTCAGAGTTCCAGG ACTGGCAAGTGTACGGTC	236	S=R=LEW=MNS=WKY=BN
DOMco6	U53910	TGCAATGTGGTCCGACTC TGTGCTGCAACGATTAAG	194	S=R=LEW=MNS=WKY=BN
DOMco7	U53911	AATCCTAATTAAGACACCAC AGGTCGAGCTTACATACC	224	S=R=LEW=MNS=WKY=BN
DOMco8	U53912	CTAGAGTCGAGGCAAATG GCTCCTCTTGCTCTTGAG	226	S=R=LEW=MNS=WKY=BN
DOMco9	U53913	GATTCTCATTCTGACCACATGC AGAGAGCACCAATAATGCTTCG	200	S=R=LEW=MNS=WKY=BN
DOMco10	U53914	GTCGTTCTTGAGGCTTTTGAGG ATGATGGATGTTGTCTCAGG	298	S=R=LEW=MNS=WKY=BN
DOMco11	U53915	GAGAAGTCCTCTGAGAGTTTGG CTAAGCTATCCTTTAGTCCTGG	299	S=R=LEW=MNS=WKY=BN
DOMco12	U53916	TGGGAATTGAACTCAGAACCTT ATGGATGGAACTAGAAAATACC	235	S=R=LEW=MNS=WKY=BN
DOMco13	U53917	CACAAGCCCTAAATTAAGTG ATCAGTCTGCCACATCTG	203	S=R=LEW=MNS=WKY=BN
DOMco14	U54500	TGTACTGAGCCATCTTCTTAGC GAGTTGAGGTCCATTCTGTAGG	249	S=R=LEW=MNS=BN>WKY
DOMco15	U53918	GCTCCCTCAAGTCATTCTGTCAAG GTGGCAATCAATAAGCAAATGGA	195	S=R=LEW=MNS=WKY=BN
DOMco16	U53919	ACCTGAATGAAGCCTGTC ACTCGAGTCCTGAATGTG	265	S=R=LEW=MNS=WKY=BN
DOMco17	U53920	GAGGTGGGTGATGTGGTA AAGTGAGGGTTTTTGAGTGA	188	S=R=LEW=MNS=WKY=BN
DOMco18[a]	U53521	GCAGTGGGCTCAATG TGGTACTTTTCACTGGTTT	312	MNS>BN>LEW=S=R=WKY
DOMco19[a]	U53522	AGGCTCGTGGTCTCATTATC AACCCTCGTCTCCTTCTTTT	269	LEW=WKY>BN=S=R>MNS
DOMco20[a]	U53523	AGTGATTCATGTGGTGGCTGTT GACTTCTCGCCAACTCAGAGTG	208	MNS=WKY=BN>S=R=LEW
DOMco21[a]	U53524	GCACTTAACATTATATGGACTC TTCACAATGCTGGTAAAGT	243	LEW=MNS=WKY>S=R=BN
DOMco22	U54501	CGAGGTTCCAATGTGTCC CGGGCGGCAATGTGGTA	314	S=R=LEW=MNS=WKY=BN
DOMco23	U54502	CGAGCCTACACAGAGAACTAC CGATCGAGACCTAAATGTG	211	S=R=LEW=MNS=WKY=BN
D5Mco1	U54616	TTGCTATTTGAGACAGGG CCCTTACTGGAGAAAGTG	253	S>MNS=BN
D10M11Mit168[b]		Research Genetics		S=R>LEW=MNS=WKY
D10M11Mit221[b]		Research Genetics		MNS=BN=WKY>S=R>LEW
D10M11Mit222[b]		Research Genetics		WKY>LEW>S=MNS=BN=R
D10Arb305	U12243	Zha et al. 1995		LEW>WKY>R=MNS=BN>S
D10Mco1	U53217	TTGACCACAGTCCACTATG GGTGAAGTTGGTCTCCTG	187	LEW=MNS>WKY=BN>S
D10Mco2	U53503	GATGTGGCAGATGTTGTC ATGTGGAGGAAAGAAACC	237	S=BN>MNS>LEW=WKY
D10Mco3	U53504	TGCCTATGATGTGGAAAG ACTCTACTGCAGCTTTGG	249	BN>S=LEW=MNS=WKY
D10Mco4	U53349	CAGCCTCAGAAGATCCAG GCAGCTGAAAGCATAGTG	200	LEW>WKY>MNS>S=R>BN
D10Mco5	U53350	AATAGACAGTGCCCAAAGAATG TCCAACCTCACGTCAAAATACT	176	WKY=S>R=MNS>LEW>BN
D10Mco6	U53351	ACTGAGTGCAAACAAAGC CCATGAGTCCTGTATCCC	187	WKY>MNS=LEW=BN>S=R
D10Mco7	U53352	ATTGGTGGCATATGCGTGTGAT CAGGATGCATGCAGGACTTTGT	314	S=R=LEW>WKY>BN>MNS
D10Mco8[c]	U53353	GAGGGAAGAATTGATGCACACG AACCATCTCTCCAACAACAACC	321 (145)	BN>S=WKY>R=LEW=MNS
D10Mco9	U53354	CCCTGCAAGTGCCTTTATG ACGAGGTGGAGATGTGGGTTC	190	LEW=WKY>MNS>S=R=BN
D10Mco10	U53355	GAGAGGTCACAGGGGTTGC TATGCCGTTAATGTGTTGTTTG	199	S=R>LEW>MNS=WKY>BN
D10Mco11	U53356	CCCACATGAAGACTTACAG TGTTCTTAACCGTTGAGC	251	LEW=S=MNS=WKY>BN
D10Mco12	U53357	CGAGCTAAACATGTGGGTGAAC AGGCAGAAAAAGAGGAAGATGT	263	S=R=LEW=MNS=WKY>BN
D10Mco13[c]	U53358	GTCATCATGGGGCTCACAG TGGGCATTTACACTCAGATACAT	284 (95)	S=R=LEW=MNS=WKY>BN
D10Mco14	U53359	AAGTATGTGGTTGGTAAATG TGTGTATAGTGTGTAGTAAGTG	130	BN>MNS>S=R=LEW=WKY
D10Mco15	U53360	GCTGGCATGGGCTACTTA GCCAGGGCTATATCGTGAGA	183	LEW=BN>S=R>MNS>WKY
D10Mco16	U53361	GGATTGGCCTAGAACTCACC GAATCCACTCCTTGTCCTCTG	186	LEW>S=BN>R=MNS=WKY
D10Mco17	U53396	TTGGGAGGAGGATGAAAGACACC CTGGGAAGGGCGACTATCAATG	366	BN>S=R=WKY>LEW>MNS
D10Mco18	U53397	GTGGGTGCCGAGAACAGAACAT ACTCCAGCTCCAAGGGTCACACT	156	MNS>BN>LEW=WKY>S=R
D10Mco19[c]	U53398	ACCACAGTCAAGGCATTCTCTAC GATCAGTGTGCCTCGGTCTTTTA	394 (180)	BN>S=R=LEW=MNS=WKY
D10Mco20	U53399	TTGGCTCCCTCCCTATTTCTTT GATGCAGGCTTTTAGGACAGTTG	234	S=R>BN>LEW=MNS>WKY
D10Mco21	U53400	TGTAGCAAGCTGGTTAGGATGTT GATGGTAGGGCAGTTAAGAAAGTG	206	MNS>WKY>S=R>BN>LEW
D10Mco22	U53401	AGCATCTGGGGACTTTACTTTC GCTTGGCCTAGGTGACAGA	263	MNS>BN>S=R=LEW>WKY
D10Mco23	U53402	CCTGTGGGAATCTACTTTGA ATTTGATGGGGATACTCTACAG	187	BN>S=R=WKY>LEW=MNS
D10Mco24	U53403	CCTGCCCCTGAGCCTGACTATCT TGCTGCCATGCTCCCACCTT	313	WKY>BN>LEW>MNS>S=R
D10Mco25	U53404	GTACAACTCAAACACAAG TATATCAGAGTAATGCTAAC	280	S=R=LEW=MNS=WKY>BN
D10Mco26	U53405	AGACATTTTCCCATCCTTTCAC CTACCAATCCAGCCCAACAG	275	S=R=LEW=MNS=WKY>BN
D10Mco27	U53406	TGCTCCACAAAAGACCTACAC AGCATGCTCTTCTGACCTTACT	133	BN>S=R=LEW=MNS=WKY
D10Mco28	U53407	GTTGATGTGCATTATTCG CCACCTACTACCCTTAACC	208	LEW=BN>WKY>S=MNS>R
D10Mco29	U53907	ACGGCCCGGGCTGGTCTG GGTGAGGTGCCCGAGGTAGTATGG	177	S=R=LEW=MNS=WKY=BN
D10Mco30	U53908	ACGATTTTCTGTCCCTTGTCTGAG TGGTTTAGCTAGGTGTGGCATCTG	257	S=R=BN>LEW=MNS=WKY
D10Mco31 (Ngfr)	U59296	CCAGCTTGAGGGTCTGAAATGT GGCAACAAGATTTTTTGGGGGC	255	LEW=MNS>S=WKY=BN
D10Mco32	U59319	CAGGCCTGGGGTTAGTAGTGAG GGAGGCCCCAGTTTGAGAG	191	S=R=BN=LEW=MNS=WKY
D10Mgh6		Research Genetics		MNS>LEW>WKY>BNS>S=R
D10Mgh5		Research Genetics		LEW>WKY>S=R=MNS=BN
D10Mgh8		Research Genetics		MNS=BN>S=R>WKY>LEW
D10Mgh10		Research Genetics		BN>R=LEW=WKY>MNS>S
D10Mgh12		Research Genetics		LEW=WKY>S=R>BN>MNS
D10Mgh15		Koike et al. 1995		LEW>S=R=MNS=WKY=BN
D10Mit2		Research Genetics		LEW=R=MNS=WKY>S=BN
D10Mit5		Research Genetics		LEW=R>BN>S>MNS=WKY
D10Mit6		Research Genetics		BN>S=R>LEW=MNS=WKY
D10Mit10		Research Genetics		LEW=MNS=WKY>S=R>BN
D10Wox6		Univ. Oxford[d]		LEW=MNS=WKY>R=BN>S
D10Wox7		Univ. Oxford[d]		LEW=MNS=WKY=BN=R>S
D12Mco1	U53525	CAGGCTATGGAATTGTTTGTGT GTGAGTGATTTGGGTCTGTGC	197	S=R>BN>LEW=MNS=WKY
RNACRB1		Kreutz et al. 1995		BN>LEW=WKY>S=R=MNS

[a]Polymorphic markers from Chr 10 sorted DNA, but unlinked to the rat Chr 10.
[b]Mouse markers, newly developed for rat Chr 10.
[c]For resolving fragments after PCR with labeled primer (forward for *D10Mco8* and reverse for *D10Mco13* and *D10Mco19*), additional restriction with *Hha*I, *Dra*I, and *Eco*R1, respectively, was performed. The size of the relevant restriction product is shown in parentheses.
[d]ftp://ftp.well.ox.ac.uk/pub/genetics/ratmap.

Ace and *Nos2* with conserved synteny to rat Chr 10 were tested. Two of these markers were informative. *D11Mit221* is polymorphic between both S and MNS and S and LEW; *D11Mit222* is polymorphic only between S and LEW. These markers are renamed for the rat *D10M11Mit221* and *D10M11Mit222* respectively (Table 1). An additional mouse marker, *D10M11Mit168,* was placed on the rat map distal to the *Ace-Nos2* region (Fig. 1).

Construction of linkage maps for rat Chr 10. MAPMAKER/EXP was used to construct linkage maps (Fig. 1) for three populations: F_2(S × MNS) (n = 171), F_2(S × LEW) (n = 151), F_2(S × BN) (n = 96). An additional composite map was constructed in which data for all three populations were combined (Fig. 2). The Kosambi mapping function was used. The genetic map for F_2(S × MNS) spans 82.5 cM and consists of 47 markers; F_2(S × LEW) spans 88.6 cM and consists of 23 markers; F_2(S × BN) spans 86.6 cM and consists of 18 markers. The composite map spans 88.9 cM and consists of 61 markers (51 anonymous markers and 10 gene loci). The average distance between markers is 1.46 cM. No markers are separated by more than 6.8 cM. Six loci are common to all three population maps, and 15 markers are common to at least two maps.

Chr 10 congenic strains. Four congenic strains [S.M(10a), S.M(10b), S.M(10c), S.M(10d)] were produced by introgressing regions of Chr 10 from MNS rats into the S recipient strain. In the names of the congenic strains, S refers to the recipient inbred Dahl SS/Jr strain, followed by a period, followed by M for the donor MNS strain. The numbers/letters in parentheses are arbitrary designations, where 10 refers to Chr 10, and a, b, c, d are arbitrarily assigned to each strain. The regions transferred are shown in Fig. 3. The markers defining the crossover region at the ends of each congenic strain are given in Table 2 because some of them cannot be depicted accurately in Fig. 3.

Table 3 gives comparisons between the congenic strains and S rats for blood pressure and heart weight/body weight ratio. The blood pressure deviation of a congenic strain from the S is also presented in bar graph form on the lower part of Fig. 3. It is clear that the S.M(10a) and S.M(10b) congenic strains include a blood pressure QTL, since these strains had blood pressures that were

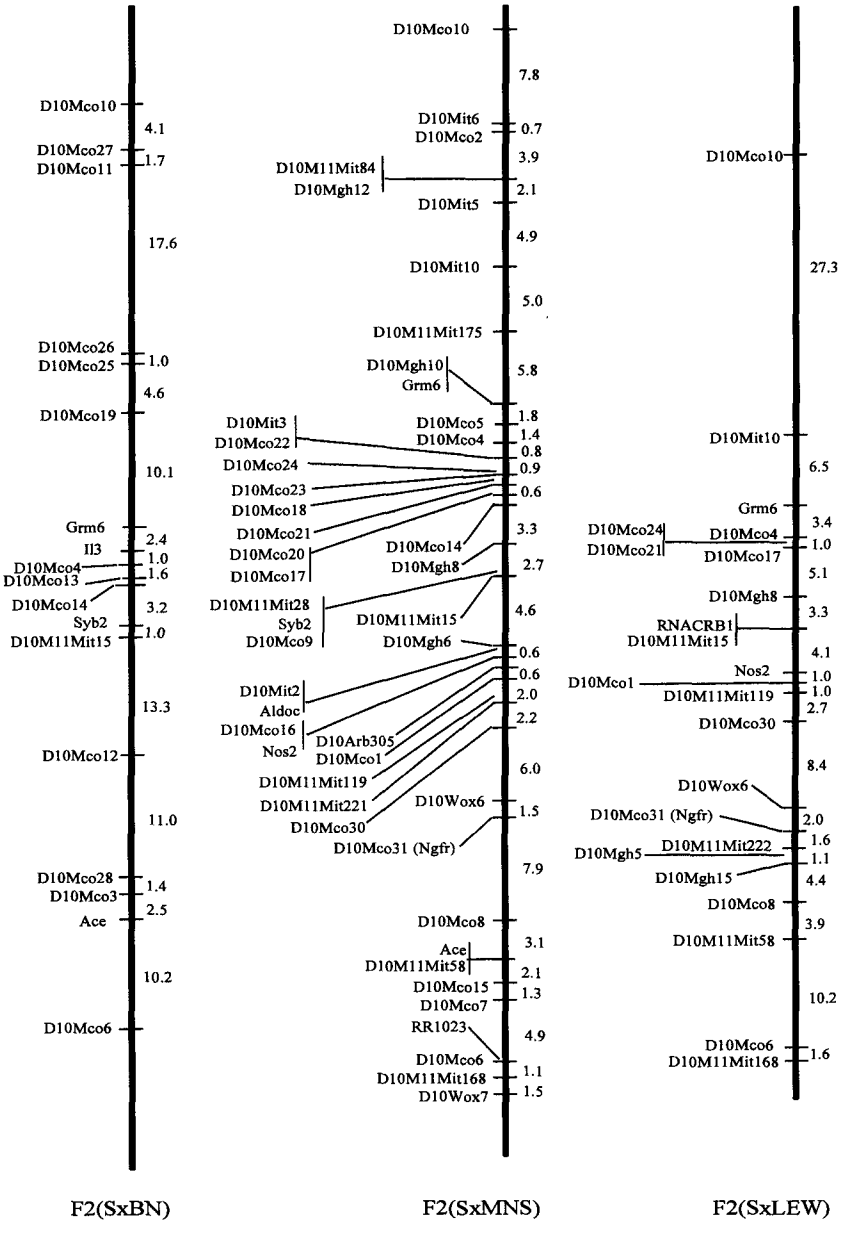

Fig. 1. Chromosome 10 maps for three crosses: F_2(S × BN), n = 96; F_2(S × MNS), n = 171; F_2(S × LEW) n = 151. Genetic markers are either listed in Table 1 or were published previously (Deng and Rapp 1995). Numbers are map distances in cM, by use of the Kosambi mapping function.

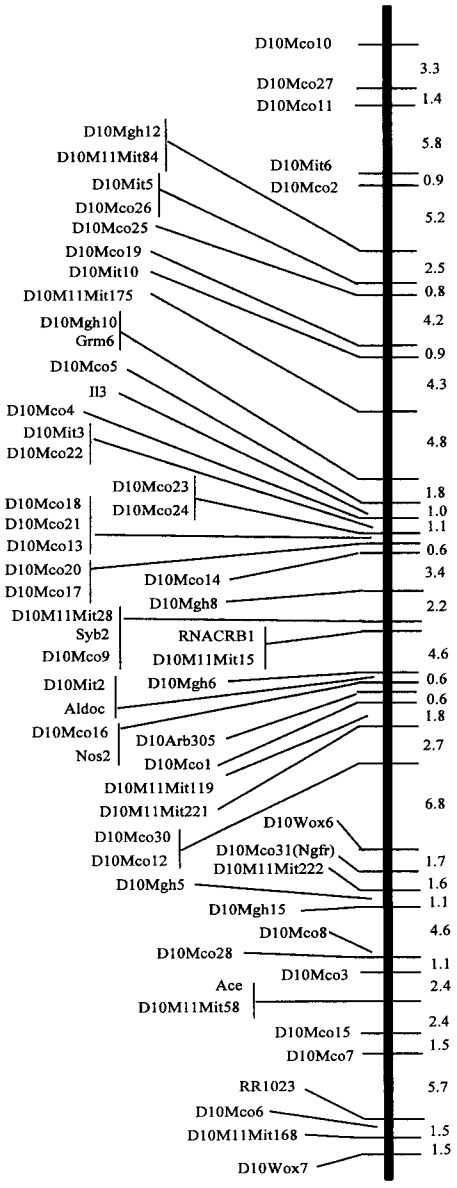

Fig. 2. Composite Chr 10 map derived from the three maps in Fig. 1. The centromere is at the top end of the map (Simon et al. 1996). Numbers are map distances in cM with the Kosambi mapping function.

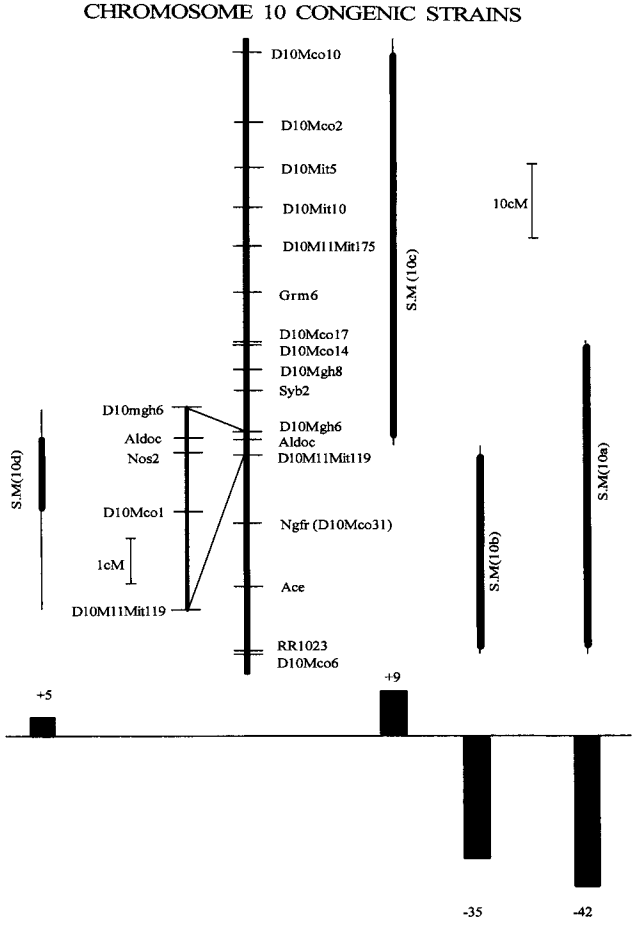

Fig. 3. A map of selected markers on rat Chr 10 is shown along with bars defining the chromosomal segments introgressed from MNS rats into the S strain in the construction for congenic strains S.M(10a), S.M(10b), S.M(10c), S.M(10d). All selected markers on the map were genotyped in defining the introgressed segments. The thin lines at the ends of the bars represent the regions of ambiguity in which crossovers occurred; the markers defining these regions are given in Table 2. The lower part of the diagram gives a bar graph indicating in mm Hg the deviation of blood pressure of the congenic strain from the recipient (control) S strain.

significantly reduced respectively by 42 ± 4.3 (SE) and 35 ± 4.5 mm Hg ($p < 0.0001$) compared with S rats. These blood pressure differences were corroborated by lower heart weight/body weight ratios ($p < 0.0001$) in the S.M(10a) and S.M(10b) congenic strains compared with S.

The congenic strains S.M(10c) and S.M(10d) showed minor changes in blood pressure, with the congenics being 9 ± 4.7 and 5 ± 3.7 mm Hg higher than S. These smaller blood pressure changes did not result in changes in heart weight/body weight ratio.

Discussion

Using rat chromosome-sorted DNA, 28 new markers were mapped to Chr 10. While the distribution of these new markers was good overall, spanning approximately 86 cM, there are regions in which we were unable to find new markers (that is, the 16-cM region between *D10Mco8* and *D10Mco12*) and one region, between *D10Mco14* and *D10Mco4*, in which ten new markers are clustered in 2.5 cM. This could be owing either to the uneven distribution of microsatellites along Chr 10 or preferential amplification of some regions of the chromosome during degenerate oligonucleotide primed PCR amplification of chromosome-sorted DNA prior to cloning. The percentage of sequences that turned up more than once in libraries derived from chromosome-sorted DNA (33%) lends support to the possibility that some regions of Chr 10 are preferentially amplified by degenerate oligonucleotide primed PCR.

Commonly accepted estimates for the abundance of $(CA)_n$ repeats suggest that, on average, they should be found every 30,000–50,000 bases. However, there are indications that microsatellites

Table 2. Markers defining the crossover regions at the ends of Chr 10 segments transferred from MNS to S in construction of congenic strains.

	Crossovers between markers	
Congenic strain	Proximal	Distal
S.M(10a)	*D10Mco17/D10Mco14*	*RR1023/D10Mco6*
S.M(10b)	*D10Mco1/D10M11Mit119*	*RR1023/D10Mco6*
S.M(10c)	Outside Map/*D10Mco10*	*Aldoc/Nos2*
S.M(10d)	*D10Mgh6/Aldoc*	*D10Mco1/D10M11Mit119*

Table 3. Comparisons of the S strain and congenic strains with substituted segments of Chr 10 from MNS on the S background.

	Blood pressure (mm Hg)	Heart Wt./ Body Wt. Ratio (mg/gm)	N
Experiment I:			
S	217 ± 4.0	4.30 ± 0.061	18
SM(10a)	175 ± 1.7	3.80 ± 0.034	20
SM(10b)	182 ± 2.2	3.84 ± 0.043	20
One-way ANOVA	<0.0001	<0.0001	
S vs S.M(10a)	<0.0001	<0.0005	
S vs S.M(10b)	<0.0001	<0.0005	
S.M(10a) vs S.M(10b)	NS	NS	
Experiment II:			
S	205 ± 2.9	3.95 ± 0.054	20
S.M(10c)	214 ± 3.6	4.06 ± 0.048	20
t-test	0.016	NS	
Experiment III:			
S	205 ± 2.6	4.06 ± 0.043	41
S.M(10d)	210 ± 2.7	4.09 ± 0.039	38
t-test	NS	NS	

Rats were fed 2% NaCl diet for 24 days starting at 40 days of age. Data were analyzed by a one-way analysis of variance (ANOVA) in experiment I followed by contrasts using Scheffe's method. In experiments II and III, the S and a congenic strain were compared by a *t*-test. NS = not significant; i.e., $p > 0.05$. N = number of rats in each group. Means ± SE are given.

may exist in clusters. About 10% of our clones (average size under 1 kb) had more than one repeat. Further, in two attempts at chromosome walking, starting from known microsatellite markers, we successfully located additional microsatellites close to the original. A second microsatellite was found within 1 kb of *D10Mco12*, and the 3.5-kb region centering on *D10Mgh5* contains four microsatellites. Multiple microsatellites have also been found in the 4.5-kb rat pancreatic polypeptide gene (accession #M18207), which has four repeats, and the 25.8-kb rat embryonic skeletal muscle myosin heavy chain gene (accession #X04267), which has three repeats. Other examples can surely be found.

Microsatellites were associated with LINE (long interspersed repetitive elements) or SINE (short interspersed repetitive elements) sequences in a high percentage (22%) of those clones used to design primers. Six of these clones contained fragments of LINE repeats, and seven contained SINE repeats or fragments of SINE repeats (B2 or ID repeats). Primers designed from these clones frequently failed to amplify the expected product or were difficult to use for genotyping. In fact, only three primer sets in which one of the primers overlapped a repetitive element gave high-quality products. A similar observation was made by Stone and coworkers (1995) in work with bovine genomic libraries. The presence of microsatellites in some members of LINE and SINE families from a variety of mammals has already been reported (D'Ambrosio et al. 1986; Dunnen and Schoenmaker 1987; Stone et al. 1995; Duffy et al. 1996).

Rat Chr 10 maps for the F_2(S × MNS), F_2(S × LEW), and F_2(S × BN) populations (n = 171, 151, 96, respectively) are shown in Fig. 1. The large population size permits mapping, in some instances, to well under 1 centiMorgan (cM). The order of markers given along each map is the most likely order as determined by MAPMAKER/EXP. However, even for the closest markers on these maps, at least one crossover has been found to support the order given. Furthermore, the two points on the F_2 (S × MNS) map for which genotyping lends least support have been confirmed by crossovers observed in backcross breeding for construction of congenic strains. In one case, the order (*D10M11Mit168, D10Mco6, RR1023, D10Mco7*) is only three times as likely as (*D10M11Mit168, RR1023, D10Mco6, D10Mco7*). The crossover between the S and one end of the MNS portions of Chr 10 in S.M(10a) and S.M(10b) occurred between *D10Mco6* (S allele) and *RR1023* (MNS allele), confirming the map order from linkage analysis. In the second case, the order ([*Nos2, D10Mco16*], [*Aldoc, D10Mit2*], *D10Mgh6, D10M11Mit15*) is only five times as likely as ([*Nos2, D10Mco16*], *D10Mgh6*, [*Aldoc, D10Mit2*], *D10M11Mit15*). The best order was confirmed by the crossover between the S and one end of the MNS portions of rat Chr 10 in S.M(10d), which occurred between *Aldoc, D10Mit2* (MNS alleles) and *D10Mgh6* (S allele).

Previous linkage analysis has strongly supported the existence of a blood pressure QTL on rat Chr 10 in the region around the candidate loci, *Ace* and *Nos2* (Hilbert et al. 1991; Jacob et al. 1991; Deng and Rapp 1992, 1995). Our linkage analysis of the F_2(S × MNS) population (Deng and Rapp 1995) showed an unusually broad 45-cM region of Chr 10 spanning from the *Ace* to *Grm6* locus, and with a LOD score varying between 5 and 7.5 throughout this region for linkage to blood pressure. Thus, the congenic strains described here were constructed to cover large regions of the chromosome to be sure to capture the QTL. Any regions positive for the presence of a QTL can be reduced in size in a second iteration of congenic strain construction (Rapp and Deng 1995). The results show clearly that the QTL is localized in a 31-cM segment between *D10Mco6* and *D10Mco1* contained in congenic strain S.M(10b) and that the direction of change in blood pressure is congruent with linkage analysis where the MNS allele reduced blood pressure relative to the S-rat allele. This region does not include *Nos2*, thus specifically excluding *Nos2* as a candidate for the QTL on Chr 10. Congenic strain S.M(10d) was constructed specifically to test *Nos2* as a candidate and shows only a minor nonsignificant effect on blood pressure. *Ace* remains a candidate for the QTL, since it is within the segment of S.M(10b) markedly altering blood pressure. A comparison of *Ace* DNA sequences between SHRSP and WKY revealed one amino acid change in the coding sequence, but the *Ace* enzymatic properties were similar between strains (Koike et al. 1994)

The minor effect of congenic strain S.M(10c) to increase blood pressure 9 mm Hg ($p < 0.016$) over the recipient S strain needs comment. This change is in the opposite direction from the decreased blood pressure associated with the MNS chromosome in linkage analysis (Deng and Rapp 1995). Of course such an opposite effect could arise if, in fact, a minor QTL with a plus allele in the MNS, and a minus allele in the S strain, were next to the QTL on Chr 10 delineated above, where the MNS allele is minus and the S is plus. Kreutz and associates (1995) reported on a dubious "congenic" strain in which a 6-cM segment of Chr 10 from stroke-prone spontaneously hypertensive rats (SHRSP) was somehow introduced into the Wistar-Kyoto (WKY) background by genetic contamination in their colony. This 6-cM segment was deemed to affect blood pressure by about 6 mm Hg. The 6-cM segment described by Kreutz and colleagues is contained in the distal end of S.M(10c) near *D10Mgh6*. In our view, these results by Kreutz and coworkers and the minor effects associated with S.M(10c) in our work do not establish clearly meaningful effects on blood pressure that are worth pursuing. In contrast, the results with our congenic strains S.M(10b) and S.M(10a) do establish a region containing an important blood pressure QTL that can be dissected further.

The magnitude of a QTL effect on blood pressure is often highly dependent on genetic background. In our linkage analyses, larger blood pressure effects were often seen in segregating populations with a higher percentage of S-rat genes in the genetic background; for example, a backcross to S, as opposed to an F_2 population (Rapp et al. 1990; Cicila et al. 1993, 1994). Thus, we suggest that in making congenics it is best to put the low-blood-pressure allele on the hypertensive background rather than putting the high-blood-pressure allele on the normotensive background. In this context it is not surprising that the Chr 10 QTL effect appears greater in the congenic strains on the full S-rat background (35–42 mm Hg, 2% NaCl diet for 3.5 weeks) than in the previously reported F_2(S × MNS) population with 50% S-rat background (22 mm Hg on 8% NaCl diet for 10 weeks; Deng and Rapp 1995).

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