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Alleles of the Spontaneously Hypertensive Rat Decrease Blood Pressure at Loci on Chromosomes 4 and 13

Peter Kovács, Birger Voigt, and Ingrid Klöting
Department of Laboratory Animal Sciences, Institute of Pathophysiology, University of Greifswald, 17495 Karlsburg, Germany

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In this study the spontaneously hypertensive rat (SHR/Mol) and the spontaneously diabetic BB/OK rat were crossed, and the F1 hybrids were backcrossed onto the BB/OK rat in order to search for cosegregation between blood pressure and loci on chromosomes 4 and 13. Cosegregation of microsatellites on chromosomes 4 (*Spr, Npy, D4mit6, Il-6*) and 13 (*Atp1a2, D13Mit1, D13Uwm1*) with blood pressure was evaluated using one-way analysis of variance. On chromosome 4 linkage of the *Npy* and *D4Mit6* markers to systolic blood pressure was observed. A blood pressure QTL was also found on chromosome 13 within the renin locus. Surprisingly, alleles of the SHR strain at loci showing linkage to blood pressure on chromosome 4 and 13 promote lower blood pressure than the same alleles from the BB/OK strain. The transfer of *D4Mit6* and renin locus from the SHR rat onto the genetic background of BB/OK rat will probably not lead to a model of diabetic hypertension, but the thorough characterisation of such congenics could contribute to the explanation of genetics and pathophysiology of hypertension in the SHR rat.

The diabetes-prone BB rat is a well-established animal model of insulin-dependent diabetes mellitus. The animals develop an autoimmune diabetes closely resembling the human disease, including rapid onset, autoimmune destruction of pancreatic islet cells, autoantibodies to β cell components and the requirement for daily injections of insulin(1). Despite close resemblance there is also a marked difference to human diabetes. In contrast to human diabetics, the BB rat does not develop the diabetic hypertension and nephropathy, which markedly determine the morbidity and mortality of diabetic patients. One supposes that the BB rat is not genetically susceptible for these diseases(2-3). To overcome this problem the spontaneously hypertensive rat (SHR) may be used to transfer chromosomal regions with quantitative trait loci (QTLs) for blood pressure onto the genetic background of diabetes-prone BB rats. With this procedure congenic BB.SHR rat strains could be generated to study not only diabetic hypertension but also the physiologic importance of putative QTLs.

The genetics of hypertension in SHR rats is complex, and the studies using either candidate gene or genomic screening approach have revealed more than 10 blood pressure loci including renal associated genes(4-13). However, the results of cosegregation studies are strain-dependent (14) and the previously described blood pressure loci are not necessarily the same as those which exist in a cross of BB and SHR rats. The aim of this study was to search on chromosomes 4 and 13 for loci that affect blood pressure. These could be appropriate candidates for chromosomal transfer onto the genetic background of diabetes-prone BB rats. Therefore F1 hybrids derived from commercially available SHR/Mol and our well-characterised diabetic BB/OK rats(15) were backcrossed onto the BB/OK rats and the backcross population BC1BB was analysed for cosegregation of blood pressure with microsatellites, mapped previously on rat chromosomes 4 and 13 by Serikawa et al.(16) and Jacob et al.(17).

MATERIALS AND METHODS

Experimental animals. Male and female diabetic, but normotensive BB/OK rats(15) were reciprocally crossed with commercially available hypertensive, but nondiabetic SHR/Mol rats (Mollegaard Breeding Centre Ltd, Denmark). The F1 hybrids were backcrossed onto male and female BB/OK (BC1BB) and SHR (BC1SHR) rats as described recently(18). The animals were kept separately in groups of 3 in Macrolon cages (Size 3, Ehret GmbH, Emmendingen, Germany) under strict hygienic conditions and were free of major pathogens as described previously(15). They had free access to food (Ssniff R, Soest, Germany) and water.

Phenotypic characterisation. From the 32nd to the 34th week of life the systolic blood pressure was repeatedly measured in conscious restrained male rats three separate times between 9:00 and 11:00 a.m. by the tail-cuff method (Kent Scientific Corporation, Kent, England) as described in detail previously(19). All measurements were carried out by the same person.

Genetic typing by polymerase chain reaction (PCR). Seven microsatellites on chromosomes 4 (*Il-6, D4Mit6, Npy, Spr*) and 13

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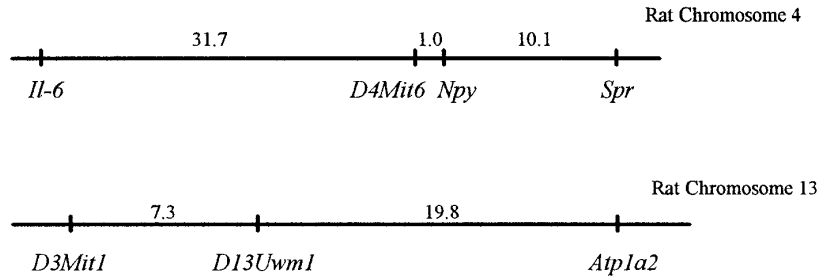

FIG. 1. Linkage map of rat chromosome 4 and 13 using microsatellite markers of Serikawa et al. (16) and Jacob et al. (17). The distances between markers are in cM, computed by MAPMAKER/EXP 3.0b.

(*D13Mit1, D13Uwm1, Atp1a2*), which were polymorphic between BB/OK and SHR/Mol rats(19), were used to genotype the cross hybrids. Amplification programs and the PCR reactions were performed as described previously(20). Primer sequences were provided by Serikawa et al.(16) and by Jacob et al.(17).

Data analysis. Because the F1 population indicated that hypertension was inherited in a dominant manner, the BC1BB male population was used for cosegregation analysis. Cosegregation of blood pressure with alleles at marker loci on chromosomes 4 and 13 was evaluated by comparing blood pressure among genotypes at each locus by one-way ANOVA using SPSS programs. In order to create a genetic linkage map, the backcross populations were combined and an F2 population was constructed, which was comprehending an allele proportion of 1 BB/BB : 2 BB/SHR : 1 SHR/SHR. The genetic linkage map was computed using the MAPMAKER/EXP 3.0b computer program(21).

RESULTS

As shown in Fig. 1, the genetic markers polymorphic between BB/OK and SHR rat strains span approximately 43 cM on chromosome 4 and 27cM on chromosome 13. The maps were in accordance with those of Jacob et al.(17).

The results of one-way analysis of variance indicate the significant cosegregation of *Npy* and *D4Mit6* markers on chromosome 4 and of *D13Uwm1* (renin) on chromosome 13 with systolic blood pressure (Table 1). The allele frequencies were statistically not different from the expected ratio - 1(BB):1(BS). The Table also shows the systolic blood pressure of each of the two genotypes segregating at investigated loci on chromosomes 4 and 13. Blood pressure effect (the blood pressure of rats homozygous for BB/OK alleles [BB] minus the blood pressure of rats heterozygous for BB and SHR alleles [BS]) at these loci was positive and reached maximum values 10.5 mmHg on *Npy* and *D4Mit6* and 10.1 mmHg on the renin locus. Surprisingly, the blood pressure effect at these loci was positive, which means that the SHR alleles promote lower blood pressure than the same alleles in BB/OK rats.

DISCUSSION

Our study showed linkage of loci on chromosomes 4 (*Npy, D4Mit6*) and 13 (renin) to blood pressure using a new set of backcross progenies derived from SHR and BB/OK rats. The study of Katsuya et al.(9) showed cosegregation of the neuropeptide Y locus on chromosome 4 with blood pressure using F2 hybrids derived from SHR and normotensive WKY rats. In contrast, no significant association between *TaqI* restriction fragment length polymorphisms of *Npy* gene and the pres-

TABLE 1

Cosegregation Analysis of Systolic Blood Pressure (mm Hg), with Alleles at Loci on Rat Chromosome 4 (*Il-6, D4Mit6, Npy, Spr*) and 13 (*D13Mit1, D13Uwm1, Atp1a2*) in the BC1BB Backcross Population (Derived from BB/OK and SHR Rats)

		Genotype			P
Chromosome	Locus	BB	BS	D	(one way) ANOVA)
4	*Il-6*	135.6 ± 19.5 (34)	136.0 ± 19.5 (22)	−0.4	0.9315
	D4Mit6	141.6 ± 21.7 (25)	131.1 ± 12.7 (31)	10.5	**0.0292**
	Npy	141.6 ± 21.7 (25)	131.1 ± 12.7 (31)	10.5	**0.0292**
	Spr	139.9 ± 20.7 (29)	131.4 ± 13.5 (27)	8.5	0.0779
13	*D13Mit1*	139.1 ± 18.7 (24)	134.1 ± 16.9 (31)	5.0	0.3077
	D13Uwm1	14.20 ± 20.1 (24)	131.9 ± 14.5 (31)	10.1	**0.0354**
	Atpla2	138.5 ± 18.3 (31)	133.4 ± 16.8 (24)	5.1	0.2936

Values are means ± SD, number of rats given in parentheses. S-allele of SHR/Mol, B-allele of BB/OK strain. D-difference in the phenotypic trait between rats homozygous for the B-allele and those heterozygous [BB-BS].

P values <0.05 are indicated in bold.

ence of hypertension has been found in human beings(22). One-way ANOVA in the backcross population in our study showed, similarly to Katsuya et al., cosegregation of *Npy* with blood pressure (Table). However, BB.LL congenics, derived from BB/OK and carrying the chromosomal region *Spr-Npy* of SHR rats, did not show changes in blood pressure comparable to BB/OK rats using telemetrical measurement of blood pressure(23). This suggests that the actual locus affecting blood pressure could map to the left of the *Npy* locus (Figure). Only the production of new congenic BB rats with the region around *D4Mit6* marker could give a definite answer as to whether there is a QTL affecting blood pressure within the transferred region, and if so, how this QTL influences blood pressure. The region between *D4Mit6* and *Il-6* spans about 32 cM so that additional polymorphic markers in this region are essential to localise this new blood pressure QTL on chromosome 4 more accurately.

Regarding the importance of the renin gene in blood pressure regulation there are several studies indicating that genetically determined variation in renin gene expression can affect blood pressure, but the results are partially controversial. In F2 populations obtained by crossing of Dahl salt-sensitive with Dahl salt-resistant rats, the animals homozygous for the renin allele of Dahl salt-sensitive rats had higher blood pressure than those homozygous for the renin allele of salt-resistant rats used for crossing(24). In contrast, the chromosomal transfer of the renin allele of the Dahl salt-sensitive rats onto the genetic background of Dahl salt-resistant rats indicated that the blood pressure of this newly established congenic rat strain was significantly lower than that of the progenitor Dahl salt-resistant strain(25). Also, the cosegregation analysis in the BC1BB population in our study showed that the region within the renin locus decreased blood pressure significantly in BS heterozygous rats in comparison with homozygous BB rats. Whereas a cosegregation of renin gene genotypes with blood pressure were also observed by Kurtz et al.(26) in an (SHR × Lewis)F2 population, no linkage of renin genotype to blood pressure was observed by Lindpaintner et al.(27) in (stroke-prone SHR × WKY)F2 hybrids. The study of Samani et al.(28) with (SHR × WKY)F2 showed the strongest cosegregation of blood pressure with the marker *D13Mit2,* and not with the renin gene. *D13Mit2* was mapped 21.7 cM away from the renin locus, but there was a suggestion of multiple peaks. These findings, including ours, confirm that the cosegregation results with blood pressure for a candidate locus are not consistent among different crosses and have to be evaluated in the appropriate cross.

It seems to be a paradox that SHR alleles of renin gene and *D4Mit6* markers promote lower blood pressure than those of the BB rat. However, hypertension in the SHR rat is the result of the net effect of multiple genes on blood pressure, some of which may promote increased blood pressure, some of which may promote decreased blood pressure(25). Thus, the fact that the blood pressure of SHR rats is greater than that of BB/OK rats does not mean that every blood pressure gene in the SHR strain causes greater pressure than the genes in BB/OK. So, it is not surprising that the SHR renin and *D4Mit6* alleles promote lower blood pressure than the same alleles in the BB/OK strain, which is consistent with results of St Lezin et al.(25), which show decreased blood presure caused by the renin allele of the hypertensive Dahl salt-sensitive rat strain. The importance of the genetic background is shown in studies of Rapp et al.(29) reporting the cosegregation of the renin allele of the Dahl salt-sensitive rat with greater blood pressure when studied on the genetic background of the Dahl salt-sensitive rat, but not when studied on the genetic background of the Dahl salt-resistant rat.

Our study showes that the transfer of chromosomal region with the *D4Mit6* (chromosome 4) or renin locus (chromosome 13) from the SHR rat onto the BB/OK rat will probably not lead to a model of diabetic hypertension. In spite of this fact, these regions remain our new candidates for a generation of congenic BB.SHR rat strains. The thorough characterisation (radiotelemetry for blood pressure measurements) of such congenics will show whether the chromosomal transfer of each of suggested regions decreases the blood pressure in BB rats and provide us new animal models, which can be further explored by physiological and pharmacological studies and which could contribute to the explanation of mechanisms involved in hypertension of the SHR rat. Of course, additional polymorphic markers between BB/OK and SHR rats in regions around the *D4Mit6* and the renin locus are essential to transfer onto the genetic background of BB/OK rat the smallest regions possible.

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