SHR.BN-Congenic Strains for Genetic Analysis of Multifactorially Determined Traits

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Abstract. The laboratory rat is an important laboratory animal with multiple well-defined inbred strains, including some of the most widely used animal models of human diseases. Recent advances in the development of rat genetic resources will enable the exploitation of the full potential of rat models of human diseases and, in addition, the rat can provide useful information for comparative genomics of humans and mice. In the current review, we describe the development of congenics strains by introgression of differential chromosome segments from the Brown Norway (BN) rat to the genetic background of the spontaneously hypertensive rat (SHR). These SHR.BN-congenic strains and recombinant sublines derived from them were developed as a model system for genetic analysis of multifactorially determined pathophysiological and morphological conditions.

The laboratory rat (*Rattus norvegicus*) is an important animal model for physiological and biochemical studies. Over 200 well-defined inbred strains are available in this species (Hedrich 1990), including some of the most widely used animal models of human diseases. In addition, thanks to the recent advances in the development of rat genetic resources, these strains will enable the exploitation of the full potential of rat models of human diseases and they can provide useful information for comparative genomics of humans and mice. Genomic studies and comparative studies in model organisms will be absolutely indispensable for deciphering genome sequences, aiming from gene identification to gene function determination. Recent advances in rat genetics include development of two new radiation hybrid maps, which contain thousands of new gene markers (Steen et al., 1999; Watanabe et al., 1999); altogether, the integrated maps provide reference to more than 3000 genes and expressed sequence tags (ESTs) and more than 8500 anonymous genetic markers. As noted by Nadeau (1999), the progress in the development of rat gene resources was so rapid that *Rattus norvegicus* is suggested to be admitted to the "G7 (Genome 7) community".

Over 98% of the most frequent human diseases belong to multifactorially determined pathophysiological conditions. Such traits can be often defined quantitatively, and the responsible loci are therefore referred to as quantitative trait loci (QTL). Identification of these QTL is, however, very complicated, because in such complex traits there is no direct relationship between a genotype and a pathophysiological phenotype, since the compensatory mechanisms can effectively modify any perturbations to the status quo and the initial defect is not apparent. To be able to genetically dissect such complex traits, it is necessary to map the putative QTL to specific chromosomes using genetically segregating populations derived from established rat models. Such linkage studies represent the first necessary step in QTL identification; however, they enabled localization of responsible genes only to relatively wide chromosome regions. The next step, after such localization of QTL in linkage studies, is its genetic isolation within a differential segment of congenic strains. This provides definitive evidence that a gene or genes with effects on a given phenotype are located within the implicated chromosome segment. Since the confidential intervals for mapping QTL in linkage studies are usually quite large (Darvasi et al., 1997), the congenic strains are typically derived by transferring relatively long regions of chromosomes to provide a reasonable chance that the QTL will be trapped within the differential chromosome segments. Various strategies can then be used in the follow-up studies to pinpoint the responsible QTL that have been genetically isolated in the congenic strains.

In the current review, we describe the development and exploitation of model systems for genetic dissection of hemodynamic, metabolic, and morphologic complex traits. These models were derived from (1) the most widely used animal model of human essential hypertension, the spontaneously hypertensive rat (SHR) (Okamoto and Aoki, 1963), and from (2) the Brown Norway (BN) congenic strain (BN-*Lx*) which carries a leg malforma-

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Abbreviations: BN – Brown Norway, ESTs – expressed sequence tags, NEFA – non-esterified fatty acid, PLS – polydactyly-luxate syndrome, RI – recombinant inbred, SHR – spontaneously hypertensive rat, QTL – quantitative trait loci.

tion mutation, the polydactyly-luxate syndrome (PLS) (Křen, 1975). The spontaneous hypertension and metabolic disturbancies are typical multifactorially determined traits (Pravenec et al., 1989). The PLS is coded for by the major gene *Lx* on chromosome 8, whose phenotypic expression is strongly influenced by modifying genes on other chromosomes (Křen et al., 1996). The QTL responsible for hemodynamic, metabolic, and morphologic phenotypes were initially uncovered by near-total genome scanning of recombinant inbred (RI) strains derived by reciprocal crossings of SHR and BN-*Lx* progenitor strains (Pravenec et al., 1989). These RI strains were described in detail in our previous reviews, including strain distribution patterns of more than 600 alleles (Pravenec et al., 1999, 1999a).

Table 1 shows the congenic strains and recombinant congenic sublines, developed by introgression of the BN or BN-*Lx* differential chromosome segments to the genetic background of the SHR strain. These congenic strains are being selected at the Institute of Biology and Medical Genetics, 1st Medical Faculty, Charles University, Prague (Cub) and at the Institute of Physiology, Academy of Sciences of the Czech Republic, Prague (Ipcv), as a model system for genetic dissection of spontaneous hypertension and accompanying metabolic disturbancies such as insulin resistance and dyslipidemia. In addition, several double congenic strains are being developed for the analysis of possible interaction of putative candidate genes in metabolic, physiologic, and morphologic traits. All congenic strains have been derived by recurrent backcrossing, and their congenic status has been confirmed by multiple polymorphic markers dispersed throughout the rodent genome.

Blood pressure analysis

Congenic strains are genetically identical with the progenitor strain except for a single chromosome segment. If such congenic strains show a difference in blood

Table 1. SHR.BN-congenic strains

Congenic Strain[1]	RNO	Donor Strain	Generation	References
SHR.BN-D1Wox6-D1Mgh11/Ipcv	1	BN/Cr	N10F10	St. Lezin et al., 1997
SHR.BN-Igf2/Ipcv	1	BN/Cr	N10F3	St. Lezin et al., 2000
SHR.BN-D2N91-Fgg/Ipcv	2	BN/Cr	N10F5	(unpublished results)
SHR.BN-Il6-Npy/Ipcv	4	BN/Cr	N8F11	Pravenec et al., 1999a
SHR.BN-Il6-D4Mgh9/Ipcv	4	BN/Cr	N8F11	Pravenec et al., 1999b
SHR.BN-D4Mgh9-Npy/Ipcv	4	BN/Cr	N8F11	Pravenec et al., 1999b
SHR.BN-Gtg3-D5Mit10/Cub	5	BN/Cr	N10F5	(unpublished results)
SHR.BN-D8Mit5-D8Mgh6/Cub	8	BN-*Lx*/Cub	NE12F10	Křen et al., 1997
SHR.BN-D8Mit5-Kcnj1/Cub	8	BN-*Lx*/Cub	NE16F6	Křenová et al., 1997, 1999
SHR.BN-D8Mit5-Sm22/Cub	8	BN-*Lx*/Cub	NE16F6	Křenová et al., 1997, 1999
SHR.BN-D8Mit3-D8Mgh7/Cub	8	BN-*Lx*/Cub	NE16F6	Křenová et al., 1997, 1999
SHR.BN-*Lx*-D8Mgh7/Cub	8	BN-*Lx*/Cub	NE16F6	Křenová et al., 1997, 1999
SHR.BN-*Lx*-D8Mit2/Cub	8	BN-*Lx*/Cub	NE18F4	(unpublished results)
SHR.BN-Dcp1-Myh3/Cub	10	BN/Cr	N10F6	(unpublished results)
SHR.BN-D12Mit3-D12Mit5/Ipcv	12	BN/Cr	N10F11	(unpublished results)
SHR.BN-D13Mit1-D13Mgh7/Ipcv	13	BN/Cr	N10F10	St. Lezin et al., 1998
SHR.BN-D16Mit5/Cub	16	BN/Cr	N8F6	(unpublished results)
SHR.BN-Adrb2-Ttr/Ipcv	18	BN/Cr	N10F8	(unpublished results)
SHR.BN-D19Rat57-D19Rat49/Cub	19	BN/Cr	N8F6	St. Lezin et al., 1999
SHR.BN-D19Mit2/Cub	19	BN/Cr	N8F6	(unpublished results)
SHR.BN-D20Arb548-Prkacn2/Ipcv	20	BN-*Lx*/Cub	N14F >20	Křen et al., 1997
SHR.BN-Y/Cub	Y	BN-*Lx*/Cub	N9F8	Qi et al., 1999, Křen et al., in prep.
SHR.BN-D8Mit5-D8Mgh6, Y/Cub	8 + Y	BN-*Lx*/Cub	N9F6	(unpublished results)
SHR.BN-D8Mit5-D8Mgh6, D19Mit2/Cub	8 + 19	BN-*Lx*/Cub, BN/Cr	N8F6	(unpublished results)
SHR.BN-Il6-Hoxa11, *Lx*/Cub	4 + 8	BN/Cr, BN-*Lx*/Cub	N8N12F3	(unpublished results)
SHR.BN-*Lx*, Dcp1-Myh3/Cub	8 + 10	BN/Cr, BN-*Lx*/Cub	N8N12F3	(unpublished results)
SHR.BN-Agt, Ren/CubIpcv	13 + 19	BN/Cr	N8N10F7	(unpublished results)

[1]SHR/OlaIpcv strain was always used as a background strain.

pressure, this provides definitive evidence that a locus affecting blood pressure (directly or indirectly) exists within the differential chromosome segment. Using radiotelemetry measurement of blood pressure, we have found that transfers of segments of chromosomes 1 (St. Lezin et al., 1997), 2 (Pravenec et al., unpublished results), 4 (Pravenec et al., 1999b), 8 (Křen et al., 1997), 10 (Pravenec et al., unpublished results), 12 (Pravenec et al., unpublished results), 19 (St. Lezin et al., 1999), and Y (Křen et al., unpublished results) from the BN/Cr or BN-*Lx*/Cub strains to the genetic background of the SHR/Ola were sufficient to significantly decrease blood pressures. On the other hand, transfers of segments of chromosomes 5 (Pravenec et al., unpublished results), 13 (St. Lezin et al., 1998) and 20 (Křen et al., 1997) from the BN/Cr strain to the SHR caused no significant effects on blood pressure measured by radiotelemetry. When putative QTL are detected and genetically isolated in congenic strains, they can be mapped within narrower regions of differential chromosome segments using derivation of recombinant congenic sublines, as was demonstrated for instance in SHR-chromosome 1, 4 or 8 congenic strains (Table 1) (Křenová et al., 1997, 1999; Pravenec et al., 1999c; St. Lezin et al., 2000).

Insulin resistance

The spontaneously hypertensive rat is the most widely studied genetic model of human essential hypertension. In addition, the SHR displays many of the metabolic features of human syndrome X, including defective insulin action on glucose metabolism, reduced catecholamine action on lipolysis in fat cells, and dyslipidemia (Aitman et al., 1997). Thus, the SHR provides a potentially useful model for investigating the genetic basis for the association between insulin resistance and hypertension. Below, we describe two examples suggesting that insulin resistance and hypertension might be determined either by closely linked genes or even by pleiotropic effects of the same gene.

Recent linkage studies in the SHR have raised the possibility that genes influencing both blood pressure and insulin resistance exist in the vicinity of the *Il6* and *Npy* genes on chromosome 4 (Pravenec et al., 1995; Pravenec et al., 1999b). We have confirmed the existence of a gene or genes affecting both blood pressure and insulin resistance on chromosome 4 by measuring blood pressure and insulin resistance in SHR progenitor and congenic rats that are genetically identical except for a segment of chromosome 4. The circulating levels of glucose and insulin were similar between SHR progenitor rats and SHR-4 congenic rats fed the standard laboratory chow. However, in SHR progenitor rats fed a high fructose diet, serum insulin levels were much greater than in SHR congenic rats fed the high fructose diet. The serum glucose values were similar between the two groups fed the high fructose diet; however, the ratios of serum insulin/glucose in SHR and SHR.BN-Il6/Npy (SHR-4) congenic rats after administration of the high fructose diet were significantly increased in the SHR progenitor strain versus the SHR-4 congenic strain, owing to the marked hyperinsulinemia in the SHR progenitor strain. These metabolic changes were also accompanied by marked impairment in glucose tolerance in the SHR progenitor strain. The baseline glucose levels were similar in the two strains; however, after glucose loading, marked and prolonged hyperglycemia occurred in the SHR progenitor strain but not in the SHR-4 congenic strain (Pravenec et al., 1999b).

Total genome scanning of RI strains also provided evidence that QTL responsible for insulin resistance (expressed as insulin/glucose ratio) are located near the D19Mit7 marker on chromosome 19. Recently, we have observed that the SHR-19 congenic strain has significantly reduced *in vitro* phenotypes of insulin resistance: insulin-stimulated lipogenesis and glycogen production in isolated diaphragms were significantly increased in the SHR-19 congenic strain versus the SHR progenitor strain (Kazdová et al., unpublished results). Altogether, these findings strongly suggest that closely linked genes or perhaps even pleiotropic effects of the same gene on chromosomes 4 and 19 are responsible for hypertension and associated metabolic disturbances. Other congenic strains remain to be tested for phenotypes of insulin resistance.

Dyslipidemia

The SHR-4 congenic strain, which was shown to have significantly different blood pressure and insulin resistance when compared to the SHR progenitor, was also tested for abnormalities in lipid metabolism, which often accompany other cardiovascular risk factors. Previously, we have found that adipocytes isolated from SHR-4 congenic rats fed a normal diet exhibited significantly greater insulin-mediated glucose uptake and increased isoproterenol-induced lipolysis than those isolated from SHR progenitor rats also fed the normal diet (Aitman et al., 1997). In the subsequent *in vivo* studies in SHR progenitor and SHR-4 congenic rats fed a high fructose diet for 15 days, we have also found that plasma NEFA levels were significantly lower in the congenic strain than in the SHR progenitor strain. Serum triglycerides were also lower in SHR-4 congenic rats versus SHR progenitor rats (Pravenec et al., 1999b). In the SHR, a significant advance has recently been made in understanding the molecular basis for disordered carbohydrate and lipid metabolism that could shed light on the clustering of systemic cardiovascular risk factors in human essential hypertension. Given that the congenic segment of chromosome 4 was known to regulate carbohydrate and lipid metabolism in isolated adipocytes, it was elected to use cDNA microarray analysis of adipose tissue to search for genes differentially expressed in fat between the SHR

progenitor strain and the SHR-4 congenic strain. In the gene-profiling studies of adipose tissue, one particular gene was observed to show a dramatic difference in expression between the SHR progenitor strain and the SHR-4 congenic strain. No other genes showed this marked degree of differential expression (10 fold) and, therefore, attention was immediately focused on this gene. The gene was found to be *Cd36*, which mapped directly back within the differential chromosome segment. Subsequent molecular studies confirmed that the SHR progenitor strain harbors a major deletion in *Cd36* that abolished expression of the encoded protein and was responsible for the SHR abnormalities in fatty acid transport and increased circulating levels of fatty acids and triglycerides. Definitive evidence for the role of *Cd36* in the metabolism of fatty acids has been obtained by experiments using *Cd36* null and transgenic mice (Febraio et al., 1999; Ibrahimi et al., 1999).

Dyslipidemia has also been described in additional congenic strains under other dietary/environmental conditions using a high fat high cholesterol diet. It has been found that especially transfers of segments of chromosomes 13 (St. Lezin et al., 1998) and Y (Qi et al, 1999) from the BN strain to the genetic background of SHR lead to profound dyslipidemia.

Leg malformation

The leg malformation mutation *Lx* coding for the polydactyly-luxate syndrome was originally trapped in a random-bred Wistar colony, from which highly inbred PD/Cub strain (F > 60) and several congenic strains have been developed (Křen 1975, Křen et al.1995). A segment of chromosome 8 from the PD/Cub strain, carrying the *Lx* mutant allele, was introgressed to the Brown Norway strain in close linkage with Es6, Apoc3 and Ncam loci. The BN-*Lx*/Cub strain served as a progenitor strain for the derivation of BXH and HXB sets of RI strains together with hypertensive SHR strain. Eventually, also the SHR-*Lx* congenic strain was established (Křen et al., 1995, 1997). The phenotype manifestation of the *Lx* allele is distinctly different on BN and SHR genetic backgrounds. The SHR genetic background strongly suppresses the *Lx* phenotype manifestation, which is limited to the preaxial polydactyly of the hind feet in homozygotes. The PLS phenotype of the SHR-*Lx* congenic substrains, which differ in the length of the RNO8 differential segment, do not differ significantly in the morphometric measurement of the zeugopodium bones (Kemlink et al., manuscript in preparation). Double-congenic strains that carry, in addition to the *Lx* major gene, also differential segments of other chromosomes with putative *Lx*-modifiers (as assessed from the linkage analysis in BXH/HXB RI strains) are being developed to search for additional genes that interact with the *Lx*, affecting its phenotypic expression (see Table 1).

The SHR genetic background not only ameliorates the malformation affliction of the *Lx* allele, but also buffers the embryo-lethal and teratogenic effect of retinoic acid (Bílá and Křen, 1996; Bílá et al., manuscript in preparation). The SHR-*Lx* congenic strain and BXH2 RI strain were used as progenitors of a new set of RI strains complementary to BXH/HXB sets, since their PLS morphotypes represent two extremes (Křen et al., 1996). After the inbreeding of a new set of RI strains is completed, these strains will be used for the analysis of genes involved in gene – teratogen interactions (Křen et al., 1999).

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