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Genetic Analysis of "Metabolic Syndrome" in the Spontaneously Hypertensive Rat

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Summary

In the current review, we summarize results of genetic analyses of "metabolic syndrome" in the spontaneously hypertensive rat (SHR). These results include (1) linkage analyses in the HXB/BXH recombinant inbred (RI) strains derived from SHR and Brown Norway (BN-Lx) strains which revealed quantitative trait loci (QTL) for hemodynamic and metabolic traits on several chromosomes, (2) genetic isolation of these putative QTL within differential chromosome segments of SHR.BN congenic strains, (3) detailed mapping of these QTL within limited chromosome segments of SHR.BN congenic sublines, (4) sequencing of selected positional candidate genes which revealed important mutations in the Cd36 and Srebp1 SHR genes, (5) functional tests of these candidate genes in SHR transgenic lines, and (6) integrated gene expression profiling and linkage mapping in RI strains which will be used to identify co-regulated genes and to determine co-segregation of transcriptional profiles with physiological and pathophysiological phenotypes.

Key words

Metabolic syndrome • QTL • SHR • Genetics • Gene expression profiles

Introduction

The spontaneously hypertensive rat (SHR) is the most widely studied animal model of hypertension. In this strain, as in many humans with essential hypertension, increased blood pressure has been reported to cluster with other risk factors for cardiovascular disease, including insulin resistance and dyslipidemia. In the current review, we summarize our results of genetic analysis of spontaneous hypertension and metabolic derangements in the SHR.

Linkage analyses in the HXB/BXH recombinant inbred (RI) strains

Genetic determinants of spontaneous hypertension and metabolic defects in the SHR strain were localized to specific chromosome regions using

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QTL analysis of the HXB/BXH sets of recombinant inbred (RI) strains. The HXB/BXH sets of RI strains were derived by reciprocal crossings of the spontaneously hypertensive rat (SHR/Ola) and the Brown Norway (BN-*Lx*/Cub) strains (Pravenec *et al.* 1989). At present, 21 HXB and 10 BXH RI strains are available. Current map of RI strains contains more than 1000 gene markers (Pravenec *et al.* 1996, Jirout *et al.* 2003). Genetic analysis of the RI strains revealed QTL for blood pressure regulation on chromosomes 1 (Huang *et al.* 1995), 2, 4, and 19 (Pravenec *et al.* 1995), 3 (Cicila *et al.* 1994), 13 (Pravenec *et al.* 1991), and 20 (Otsen *et al.* 1996, Pravenec *et al.* 1989), QTL for heart weight on chromosomes 3 (Cicila *et al.* 1994), 12 (Hamet *et al.* 1996), 17 (Pravenec *et al.* 1995), and 20 (Kuneš *et al.* 1990), QTL for renal weight on chromosomes 1, 3, and 17 (Hamet *et al.* 1998), QTL for compensatory renal growth on chromosomes 4 (Pravenec *et al.* 2000a) and 6 (Pravenec *et al.* 1998), QTL for dyslipidemia on chromosomes 4, 10, and 19 (Bottger *et al.* 1996, 1998), QTL for insulin resistance on chromosomes 3 and 19 (Pravenec *et al.* 2002) and on chromosomes 4, 7, and 12 (Aitman *et al.* 1997), QTL for hematocrit on chromosome 4 (Pravenec *et al.* 1997), and QTL for response to stress on chromosomes 10, and 12 (Dumas *et al.* 2000).

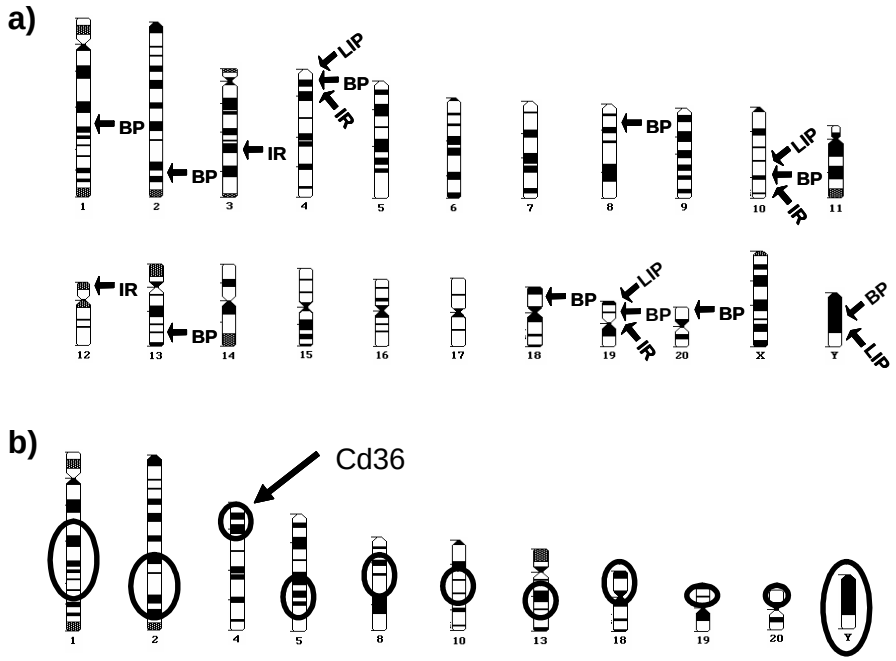

Fig. 1 Mapping and genetic isolation of QTL regulating hemodynamic and metabolic phenotypes. a) QTL associated with blood pressure (BP) and phenotypes of lipid (LIP) and carbohydrate (IR) metabolism. b) Schematically depicted differential chromosome segments in SHR.BN congenic strains in which putative QTL have been genetically isolated.

Figure 1a shows localization of QTL for blood pressure, lipid phenotypes and carbohydrate phenotypes on individual chromosomes. As can be seen, there was a clustering of cardiovascular risk factors on chromosomes 4, 10, and 19. It remains to be determined whether these clusterings are genetically determined by pleiotropic effects of a single gene or by effects of closely linked genes. As will be discussed below, variation in dyslipidemia and insulin resistance associated with a distal part of chromosome 4 is determined by a deletion mutation in Cd36 fatty acid transporter as indicated in Figure 1b.

Genetic isolation of QTL within differential chromosome segments of SHR.BN congenic strains

Putative QTL revealed by linkage analyses of RI strains were confirmed by their genetic isolation within SHR.BN congenic strains. Congenic strains were also derived for other chromosome segments in which the presence of blood pressure regulatory QTL was indicated in additional published linkage studies. Transfer of segments of chromosomes 1 (St. Lezin *et al.* 1997, 2000), 2 (Pravenec *et al.* 2001a), 4 (Pravenec *et al.* 1999),

5 (Pravenec *et al.* 2003a), 8 (Křen *et al.* 1997), 10 (Pravenec *et al.* unpublished results), 18 (Pravenec *et al.* 2001b), 19 (St. Lezin *et al.* 1999), 20 (Pravenec *et al.* 1989, Pausová *et al.* 2003), and Y (Křen *et al.* 2001a) from the BN strain onto the genetic background of the SHR were all associated with significant decreases in radiotelemetrically measured blood pressure and in some cases also with amelioration of cardiac hypertrophy. A transfer of chromosomes 4 and 19 segments was associated with amelioration of defects in lipid and carbohydrate metabolism (Pravenec *et al.* 1999, 2002), and a transfer of chromosome 13 induced dyslipidemia (St Lezin *et al.* 1998). In addition, a QTL for hepatic cholesterol concentration was genetically isolated within a new SHR.BN-chr.10 congenic strain (Pravenec *et al.* 2001c). The analysis of SHR.BN-chr.Y consomic strain provided an evidence for a QTL regulating preference to salt on chromosome Y (Di Nicolantonio *et al.* 2004). Differential chromosome segments transferred from the BN strain onto the SHR genetic background are schematically depicted in Figure 1b.

Detailed mapping of putative QTL in SHR.BN congenic sublines

Detailed mapping of genetic determinants that are responsible for blood pressure and metabolic phenotypes is being analyzed for chromosomes 4, 8, 10, and 18 in congenic sublines derived from F_2 rats obtained by crossing the original congenic strains with the SHR progenitor (Křen *et al.* 2001b, Pravenec *et al.* 2000b). Figure 2 shows the scheme of derivation of chromosome 4 sublines. Radiotelemetry measurements of blood pressures revealed significantly decreased blood pressures to the same extent in all sublines suggesting a presence of a blood pressure regulatory QTL within the D4Rat33-Npy interval (Pravenec *et al.* unpublished results).

Sequencing of selected positional candidate genes

Sequence analyses of multiple positional candidate genes revealed two unique mutations in Cd36 and Srebp1 genes on chromosomes 4 and 10, respectively. Cd36 gene codes for an important transmembrane fatty acid transporter and was identified as a prominent candidate gene because of its reduced expression in the SHR as revealed by microarray cDNA chips (Aitman *et al.* 1999). Sequence analysis revealed a deletion mutation in the SHR resulting in decreased fatty acid transport in adipose and muscle tissues, impaired insulin action, and glucose intolerance (Aitman *et al.* 1999, Hajri *et al.* 2001, Pravenec *et al.* 1999). Glazier *et al.* (2002) have found three copies of the Cd36 gene, one transcribed copy and two pseudogenes, in normal rat strains, but only a single gene in the SHR. Analysis of SHR genomic sequence localized the chromosomal deletion event between intron 4 of the normally transcribed copy of the gene and intron 4 of the second pseudogene. Serological analysis revealed that Cd36 functions as an immunogenic domain of the Rt8 alloantigen (Mlejnek *et al.* 2003).

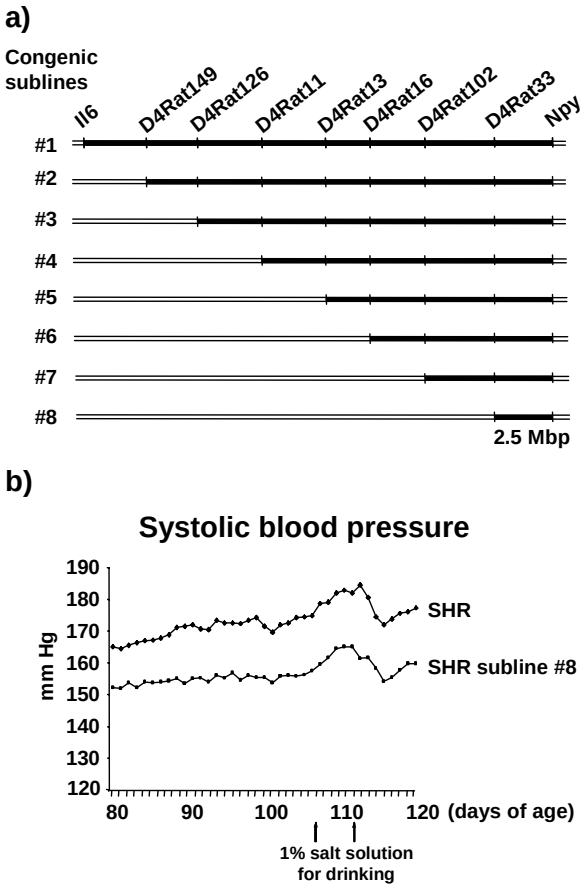

Fig. 2. Detailed mapping of blood pressure regulatory QTL on chromosome 4 using analysis of congenic sublines. a) Schematically depicted differential segments (black boxes) of chromosome 4 in congenic sublines derived for detailed mapping of blood pressure regulatory QTL. Congenic sublines were derived from an (SHR x SHR.BN-Il6/Npy)F2 intercross using polymorphic microsatellites markers to select appropriate recombinants at the boundary of each approximately 5 cM interval. b) 24 hour average systolic blood pressures measured by radiotelemetry in the SHR and SHR.BN-D4Rat33/Npy (#8) subline. The subline #8 exhibited significantly decreased systolic blood pressure.

The Srebp1 (sterol regulatory element binding protein 1) gene codes through alternative transcription start sites for two isoforms designated Srebp1a and Srebp1c that function as important transcription factors in regulation of lipid and carbohydrate metabolism (Foufelle and Ferre 2002). The SHR Srebp1 gene was analyzed as a positional candidate for hepatic cholesterol concentrations (Bottger *et al.* 1998). Sequence analysis revealed amino acid substitution mutation in the regulatory domain of Srebp1 (Pravenec *et al.* 2001c). A transfer of a chromosome 10 segment, including the Srebp1 gene, from the BN strain onto the SHR genetic background was associated with increased hepatic cholesterol and triglycerides (Pravenec *et al.* 2001d)

Functional tests of candidate genes in SHR transgenic lines

Definitive evidence for the identity of deleted Cd36 with the putative QTL on chromosome 4 was obtained by transgenic rescue experiments when the expression of wild type Cd36 under control of a universal Ef-1α promoter on the SHR genetic background that lacks functional Cd36 gene was associated with significant amelioration of metabolic defects (Pravenec *et al.* 2001e, 2003b). Relationship between Cd36 deficiency and disordered fatty acid metabolism seems to be straightforward, but it is uncertain how impaired fatty acid transport affects insulin action for instance in adipose or muscle tissues.

To assess the effects of Cd36 mediated fatty transport in specific tissues on systemic insulin sensitivity we produced a new line of SHR transgenic rats that express wild type Cd36 under control of muscle creatine kinase (MCK) promoter exclusively in muscle tissue. In these rats, Cd36 transgene expression exclusively in muscle tissue was associated with significant increases in serum triglycerides, fatty acids, and glucose. In addition, these transgenic rats exhibited impaired glucose tolerance and a decrease in antilipolytic action of insulin (Zídek *et al.* 2003). These findings indicate that expression of Cd36 exclusively in muscle tissue is not sufficient for amelioration of metabolic defects in the SHR and can actually promote dyslipidemia, insulin resistance and glucose intolerance possibly due to enhanced utilization of fatty acids at the expense of glucose utilization in skeletal muscle.

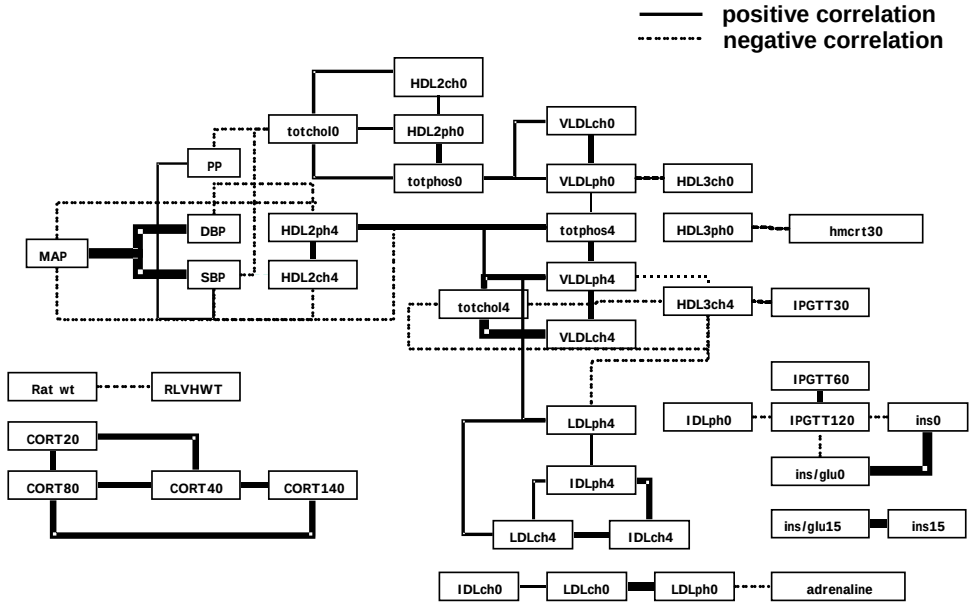

Fig. 3. Functional networks based on assays of cardiovascular traits. (SB, DB, PP - systolic, diastolic, pulse pressures; MAP - mean arterial pressure; RLVHWT - relative left ventricular heart weight; CORT20, 40, 80 and 120 - corticosterone levels 20, 40, 80, and 120 min. after immobilization stress; totchol0, HDL2ch0, HDL3ch0, VLDLch0, LDLch0, IDLch0, totchol4, HDL3ch4, VLDLch4, LDLch4, IDLch4 - total serum cholesterol and cholesterol in lipoprotein subfractions on control diet ("0") or after feeding rats 4 weeks a diet with 2 % cholesterol ("4"); phos – serum phospholipids, otherwise the same symbols as for cholesterol; hmcrt30 – hematocrit in 30 days of age; IPGTT0, 30, 60, and 120 - glucose levels 30, 60, and 120 min. after intraperitoneal glucose load; ins0 and ins15 - insulin concentrations on normal diet and after feeding a diet with 60 % fructose for 15 days; glu0 and glu15 - glucose before and after fructose feeding). The width of lines reflects the strength of statistical significance of correlations.

Experiments with Cd36 transgenic and congenic rats also provided a direct pharmacogenetic evidence that in the SHR model, Cd36 is a target gene involved in the insulin sensitizing actions of thiazolidinedione ligands of PPARγ (Qi *et al.* 2002, Šeda *et al.* 2003).

Transgenic rescue of the SHR (carrying a mutant Srebp1 gene) with a dominant positive NH_2-terminal fragment of Srebp1c under control of the PEPCK promoter, was associated with significantly increased hepatic cholesterol and triglycerides, and decreased serum triglycerides (Pravenec *et al.* 2003c). This finding strongly suggest that mutant Srebp1 underlies a QTL on rat chromosome 10 that is associated with disordered lipid metabolism in the SHR strain.

Integrated gene expression profiling and linkage mapping in the HXB/BXH RI strains

To investigate the genetic determinants of gene expression in SHR and BN rats, we have generated microarray-based expression profiles in kidney and adipose tissue from 30 RI strains and the two progenitor strains. Tissues were harvested at 6 weeks of age and expression profiles were generated for 4 animals from each RI and progenitor strain. mRNA transcript abundance for each of the 15,923 genes on the array was treated as a quantitative trait and used to map cis- and trans-acting modulators of gene expression to the rat genome. Clustering of quantitative gene expression phenotypes will be used to identify co-regulated genes and to determine co-segregation of transcriptional profiles with physiological and pathophysiological phenotypes already characterized in the RI strains. It is anticipated that, by defining the genetic networks and regulatory mechanisms underlying gene expression, we will identify many of the allelic variants that determine cardiovascular and metabolic phenotypes in these rat strains (Hübner *et al.* 2003). An example of a functional network of hemodynamic and metabolic traits determined by correlation/cluster analysis mentioned above is shown in Figure 3 (Nadeau, Pravenec *et al.*, unpublished results). This proof-of-concept study demonstrates the usefulness of RI strains for correlation and cluster analyses of physiological and expression phenotypes.

Conclusions

Studies summarized in the current review provide evidence that it is possible to identify on molecular level QTL underlying complex metabolic disorders in the SHR. It can be expected that more QTL will be identified in the near future thanks to a great progress that has been recently made in rat genetics, including availability of genome sequence of the BN strain (www.ensembl.org/Rattus_norvegicus), SNPs in coding regions of thousands of genes (Zimdahl *et al.* 2004), and microarray platforms with a capacity of over 25,000 genes and expressed sequences. In addition, use of lentiviral vectors (Lois *et al.* 2002) will facilitate production of transgenic and knockdown SHR for in vivo testing of selected candidate genes. Finally, it is quite conceivable that sequence of the SHR genome will be available in several years. These advances will greatly enhance the potential of the SHR-BN model system for the identification of responsible QTL on molecular level.

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