Metabolic Characterization of Insulin Resistance Syndrome Feature Loci in Three Brown Norway-Derived Congenic Strains

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Abstract. Studies on genetic determination of the insulin resistance syndrome in rat models revealed several susceptibility loci for features of this complex phenotype, i.e., dyslipidemia, insulin resistance and obesity. We analysed the influence of introgression of the RNO4, RNO20 segments of SHR origin and RNO8 segment of PD/Cub origin (all previously shown to be involved in (dys)regulation of carbohydrate and lipid metabolism) onto the genetic background of a common progenitor, the Brown Norway (BN/Cub) rat. The differential segments were genetically characterized in the BN.PD-D8Rat39/D8Rat35 (BN-Lx, RNO8 congenic), BN.SHAR-L6/Cd36 (BN.SHAR4, RNO4 congenic) and BN.PD-D8Rat39/D8Rat3, SHR-D4Mgh2/Cd36, SHR-D20Wox3/D20Mgh5 (BN-Lx 1K, RNO4, 8, 20 triple congenic) strains and their metabolic profiling was performed. After one week of high-sucrose diet, all congenic strains showed substantially higher levels of serum triglycerides and free fatty acids as well as impaired glucose tolerance in comparison with the BN/Cub progenitor strain. The BN-Lx 1K triple congenic strain displayed the most profound dyslipidemia, glucose intolerance and highest increase of triglyceridemia in response to high-sucrose diet overall, though accompanied with the significantly lowest adiposity index. These results further support the role of genes present within the studied chromosomal regions in observed metabolic disturbances. Furthermore, these findings point to the studied loci within the gene-gene and gene-environment interactions involved in pathogenesis of the insulin resistance syndrome. The set of defined congenic strains provides a possibility of assessing individual features of such a complex phenotype.

It is well established that the genetic component plays a major role in the development of insulin resistance, dyslipidemia, obesity and hypertension, which tend to cluster within an individual, forming a condition known as metabolic syndrome X or insulin resistance syndrome (IRS, Reaven, 1988). In the search for causal genes of such complex quantitative traits, several model rat strains have been developed exhibiting all or some features of IRS, i.e. spontaneously hypertensive (SHR) rat (Okamoto and Aoki, 1963; Aitman et al., 1999), Goto-Kakizaki (GK) rat (Goto et al., 1975), Zucker „fatty“ rat (Zucker, 1972; Bray, 1999), Otsuka-Long-Evans-Tokushima Fatty (OLETF) rat (Kawano et al., 1991, 1992), hypertriglyceridemic (HTG) rat (Vrana and Kazdova, 1990) or polydactylyous (PD/Cub) rat (Šedová et al., 2000a). Each model represents a specific allelic combination which, either alone or in combination with environmental factors such as diet, stress or pharmacological intervention, leads to the manifestation of a particular disease.

Once the chromosomal region that may contain one or more loci controlling the quantitative trait (termed quantitative trait loci – QTL) is identified, the causality of the relation between the genes present in the region to the studied phenotype must be evaluated. There are several approaches, such as candidate gene approach or production of congenic strains, each having particular advantages and limitations. Production of congenic strains (i.e. strains differing from a usually inbred progenitor strain in the chromosomal segment of interest, which had been introgressed onto the genetic background of the second progenitor) is one of the reliable
strategies of testing the relation of the locus/loci in question to the trait it/they possibly influence(s) (Rapp, 2000).

In this study, we investigated the effect of three particular regions of the rat genome previously proposed to influence parameters of carbohydrate and lipid metabolism and other features of IRS. We transferred these regions of interest from the inbred models of insulin resistance and dyslipidemia, the spontaneously hypertensive rat and the polydactylyous rat strain, onto the genetic background of the normotensive, normolipidemic Brown Norway inbred rat strain, thus creating three congenic strains.

Material and Methods

Rat strains

The Brown Norway (BN/Cub) rat strain originated from wild rats captured in the USA at the beginning of the 20th century and was made inbred by Billingham and Silvers (1959). The Brown Norway rat was transferred from the USA to the Institute of Biology, 1st Medical Faculty, in 1964 and since then bred by brother x sister mating for more than 70 generations. The BN/Cub rat strain often serves as a control strain as it was shown to be normotensive and normotriglyceridemic. In this study, BN/Cub is the common progenitor for all used congenic strains. It is of interest that the BN strain was chosen for sequencing of the whole genome, along with other model organisms (Jacob and Kwitek, 2002).

The BN.PD-D8Rat39/D8Rat35 (BN-Lx/Cub) congenic strain was derived from the BN/Cub inbred strain. The introgression of a differential segment of RNO8 of PD/Cub origin was achieved by backcross breeding onto the BN/Cub genetic background (Kfen, 1975; Kfen et al., 1995; Kfen et al., 1996). Together with a malformation mutation of the polydactylyous PD/Cub strain, the differential segment carries genes involved in lipid and carbohydrate metabolism. The BN-Lx/Cub strain thus not only represents a model of genetic determination of polydactyly-luxate syndrome, but can also serve as a useful tool for metabolic studies.

The BN.SHR-I60/Cd36 (BN.SHR4) congenic strain was derived by introgressing the RNO4 differential segment of the SHR origin onto the BN genetic background. For selection, D4Bro1 and D4Rat7 markers were used in backcross populations and Cd36/FAT with restriction endonuclease HindI in intercross populations. After nine backcross equivalents have been achieved, the test for congenicity was performed. The following markers were used: D1Mit9, D1Mit14, D1Mit27, D1Wox22, D1Mit13, D1Arb11, D1Rat46, D2Mit6, D2Mit8, D2Mit12, D2Mit16, D3Mit2, D3Mit7, D3Mgh3, D4Bro1, D4Wox16, D4Mgh4, Cd36/FAT, D4Mgh22, D4Rat142, D4Rat6, D4Rat7, D4Rat9, I66, Hoxa11, D5Mit2, D5Mit4, D5Mgh1, D5Mgh15, D6Mit2, D6Mit5, D6Mit9, D7Mit3, D7Mit12, D7Mit17, D8Mit3, D8Mit5, D8Mgh3, D8Rat44, NCAM, D8Gor72, D8Mit2, D9Mit1, D9Mit2, D9Mit4, D10Mit6, D10Mgh6, Myh3, D10Mit8, D10Mit4, D10Wox11, D10Wox12, D10Wox14, D11Mit1, D11Mit4, Sst, D12Mit3, D12Mit4, D12Mit8, D13Mit1, D13Mit2, D13Mit4, D14Mit4, D14Mit5, D15Mit3, D15Mit6, D16Mit2, D16Mit3, D16Mit5, D16Rat6, D16Rat65, D1A, D17Mit2, D17Mit4, D18Mit1, D18Mit4, D18Mit9, D19Mit2, D19Mgh3, ETA, D20Mit1, D20Mgh1, D20Mgh5, D20Wox3, DXMgh7, DXRat9, DXRat38. The status of congenicity was proved.

The BN.PD-D8Rat39/D8Rat3, SHR-D4Mgh2/CD36, SHR-D20Wox3/D20Mgh5 congenic strain, originally designated BN-Lx 1K (Pravenec et al., 1989), is in fact a triple congenic strain. Apart from the differential segment of chromosome 8 (of PD/Cub origin as in BN-Lx congenic strain), it carries a segment of chromosome 20 including the major histocompatibility complex - RT1 locus and a small segment of chromosome 4 carrying Cd36/FAT (fatty acid translocate), both of SHR origin. The chromosome 4 differential segment was found to be fixed when total genome scan of this congenic strain was performed. By total genome scan of BN-Lx 1K the congenicity of this strain was confirmed.

The following microsatellite markers were used for congenicity testing: D1Mit9, D1Mit14, D1Mit27, D1Mgh22, D2Mit6, D2Mit8, D2Mit13, D2Mit16, D3Mit2, D3Mit7, D3Mgh3, D4Bro1, D4Mit20, D4Mgh1, D4Mgh4, D4Mgh22, D4Rat6, D4Rat7, Cd36/FAT, I66, Hoxa11, D5Mit5, D5Mgh1, D5Mgh15, D6Mit2, D6Mit5, D6Mit6, D6Mit9, D7Mit3, D7Mit7, D7Mit12, D7Mit17, D8Rat11, D8Rat36, D8Rat37, D8Rat39, D8Rat44, D8Rat49, D8Rat85, D8Rat113, D8Rat127, D8Rat143, D8Rat199, D8Rat228, D8Mit6, D8Mgh4, D8Gor72, Apoc-III, D9Mit1, D9Mit2, D9Mit4, D10Mit6, D10Mgh4, Myh3, D11Mit1, D11Mit2, D11Mit4, Sst, D12Mit3, D12Mit4, D12Mit8, D13Mit1, D13Mit2, D14Mit4, D14Mit5, D15Mit3, D15Mit6, D16Mit2, D16Mit3, D16Mit5, D1A, D17Mit2, D17Mit4, D18Mit1, D18Mit4, D18Mit9, D19Mit2, D19Mgh3, ETA, D20Mit1, D20Mgh1, D20Mgh5, D20Rat17, D20Rat29, D20Rat55, D20Rat66, D20Wox3, DXMgh7, DXRat9, DXRat38.

Metabolic measurements

Male rats (3–4 months of age, 6–9 g per group) of the BN/Cub and three congenic strains were used in the experiments. The rats had free access to water and were fed standard chow followed with one week of high-sucrose diet (70% calories as sucrose). The serum concentrations of triglycerides (TG), free fatty acids (FFA), total cholesterol and glucose were determined.