Rat Inbred PD/Cub Strain as a Model of Dyslipidemia and Insulin Resistance

(metabolic syndrome X / insulin resistance / hypertriglyceridemia / PD/Cub rat strain / genetic markers)

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Abstract. Genome scan and metabolic profile of the PD/Cub rat inbred strain in comparison with SHR and BN strains is presented. The PD/Cub strain has been bred by brother sister mating for more than 60 generations since 1969. Its highly inbred status has been confirmed by PCR genotyping with more than 170 microsatellite markers. No case of residual heterozygosity has been recorded. Accordingly, the values obtained by the analysis of metabolic phenotypes are homogeneous with low variance. The PD/Cub strain has significantly higher triglyceride levels and epididymal fat weight as compared to both SHR and BN strains. The PD strain also displays the lowest incorporation of ^{14}C-U glucose into the epididymal adipose tissue. The data on glucose metabolism clearly indicate that the PD/Cub strain is insulin resistant. Genetic homogeneity and reproducibility of experimental results qualify the PD/Cub rats as an animal model for analysis of the syndrome X.

The Norway rat (Rattus norvegicus) has become an important animal model for physiological, pharmacological as well as morphological studies (Robinson, 1965; Gill et al., 1989; Hedrich, 1990). According to Steen et al. (1999), "the rat has the potential to have a major role in functional genomics and pharmacogenetics, both through studies within the organism and through comparative genomics". No wonder that rat eventually attracted the attention of geneticists and has recently been suggested to join the G7 (Genome 7) group of model organisms analyzed along with the human genome project (Nadeau, 1999).

The rat has an irreplaceable position in the analysis of genetic influences in the determination of hypertension and multiple disorders of lipoprotein and carbohydrate metabolism connected with it (Reaven et al., 1989; Reaven et al., 1996; Aitman et al., 1999; Pravenec et al., 1999a). There are several rat inbred, congenic and recombinant inbred strains exploited in the study of hypertension and other cardiovascular and metabolic phenotypes (for review see Pravenec et al., 1999b; Křen et al., 2000). The SHR strain developed by Okamoto and Aoki (1963) has been proposed as a model of the insulin resistance syndrome (Iritani et al., 1977; Reaven et al., 1989; Rao et al., 1993; Aitman et al., 1999). As is usual in biomedical research, the variability in insulin resistance was ascertained in the SHR rats from different sources (Furukawa et al., 1998). By phenotyping our collection of inbred and congenic strains we have found that the polydactylous Wistar strain PD/Cub (Křen, 1975) exhibited even higher hypertriglyceridemia and insulin resistance than the SHR strain (Vrána et al., 1993). In the present paper we are going to present the total genome scan of the PD/Cub strain, confirming its highly inbred status. Simultaneously, we are introducing new data on hypertriglyceridemia and insulin resistance of the PD/Cub strain in comparison with the SHR/OlaIpvcCub and BN/Cub strains. The high level of inbreeding and reproducibility of metabolic traits make the PD/Cub strain a useful tool for analyzing genes underlying the above mentioned phenotypes.

Material and Methods

Rat strains used in this study

PD/Cub originated from a polydactylous pair of random-bred Wistar rat strain, and since 1969 it has been mantained at the Institute of Biology, 1st Medical Faculty, Charles University, by brother sister mating (F > 60 until now). The PD/Cub strain has been exploited as a genetic model of leg malformation (Křen, 1975) and also of hypertriglyceridemia (Vrána et al., 1993). The

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Abbreviations: AA-bis – acrylamide-bisacrylamide, Apo – apolipoprotein, *Cd36/Fat* - fatty acid translocase gene, FCHL – familial combined hyperlipidemia, FFA – free fatty acids, PCR – polymerase chain reaction, QTLs – quantitative trait loci, RIA – radioimmunoassay.

spontaneously hypertensive SHR/OlaIpcvCub strain was derived by repetitive selective crossbreeding from the Wistar strain by Okamoto and Aoki (1963). It was transferred to the Institute of Physiology, Academy of Sciences of the Czech Republic, more than 20 years ago and bred simultaneously at the Institute of Biology since 1982. Brown Norway BN/Cub was transferred from the USA to the Institute of Biology in 1964 and since then bred by brother sister mating for more than 70 generations. Besides other experimental assays the BN has served as a control strain in cardiovascular research, being normotensive and having normal metabolic phenotypes.

Microsatellite genotyping

Primers for the polymerase chain reaction (PCR) detection of microsatellite markers used in characterization of the PD/Cub strain were obtained from Research Genetics (Huntsville, AL) or synthesized at the University of California, San Francisco Biomolecular Resource Center, according to known sequences. More than 170 microsatellite markers, mostly for anonymous DNA sequences and some defining known genes, were used in PD/Cub genotyping, the average number of primers used per chromosome was 4, with the exception of chromosomes 4 and 8. More detailed genotyping of chromosomes 4 and 8 was performed because congenic strains are being developed due to the finding of putative candidate genes influencing blood pressure and metabolic phenotypes on these chromosomes. After electrophoresis on 6% acrylamide-bisacrylamide (AA-bis) (19 : 1) gel and ethidium bromide staining, the PCR products were detected by Insta DocTM Gel Documentation System (Bio-Rad Laboratories, Hercules, CA), analyzed by Multianalyst (Bio-Rad Laboratories) software and printed on a thermoprinter (Mitsubishi, Kyoto, Japan).

SHR and BN rats were used as controls (1 DNA sample each) and their inbred status was repeatedly verified. PD/Cub rats (DNA samples from 2 up to 6 animals from different generations – F 45–55) were genotyped by these markers: *D1Mit9*, *D1Mit13* (defining myosin light polypeptide 2 gene), *D1Mit14*, *D1Mit27*, *D2Mit4*, *D2Mit5*, *D2Mit6*, *D2Mit21* (defining the natriuretic peptide receptor A/Guanylate cyclase A gene), *D3Mit2*, *D3Mit4*, *D3Mit7* (defining the catalase gene), *D3Mgh3* (defining the seminal vesicle protein-4 gene), *Cd36/Fat* (defining the fatty acid translocase gene), *D4Bro1* (defining the *Slc4a2* - solute carrier family 4, member 2, anion exchange protein gene), *D4Mgh4* (defining the neuropeptide Y gene), *D4Mit20* (defining the α-2-macroglobulin gene), *D4Rat7*, *D4Rat9*, *D4Rat27*, *D4Rat65*, *D4Rat72*, *D4Rat129*, *D4Rat149*, *D4Rat164*, *D4Rat222*, *D4Rat227*, *Il6* (interleukin 6 gene), *Pparγ* (peroxisome proliferator-activated receptor γ gene), *D5Mgh13*, *D5Mgh15*, *D5Mit4*, *D5Mit10*, *D6Mit2*, *D6Mit5*, *D6Mit6* (defining the creatin kinase, brain gene), *D6Mit8*, *D7Mgh9*, *D7Mgh11*, *D7Mit5*, *D7Mit7*, *Apoc3* (apolipoprotein cIII gene), *Kcnj* (potassium inwardly-rectifying channel, subfamily J gene), *Mll* (mixed-lineage leukemia), *Ncam* (cell adhesion molecule, neural (CD56) gene), *Rbp2* (retinol-binding protein 2 gene), *R850*, *D8Mcw1* defining *Sm22* (smooth muscle cell (SMC) specific protein), *Thy1* (thymus cell surface antigen gene), *Tpm* (tropomyosin 1(α) gene), *D8Arb7*, *D8Arb9*, *D8Arb12*, *D8Arb20*, *D8Arb23*, *D8Arb27*, *D8Bord1* (defining the acetylcholine receptor α 3, 5 and β 4), *D8Mgh1*, *D8Mgh3* (defining the *Rbp2* gene), *D8Mgh5* (defining the *Tpm1* gene), *D8Mgh6*, *D8Mgh7* (defining the cytochrome P-450, subfamily I (aromatic compound-inducible), member A 1 gene), *D8Mgh9*, *D8Mit2*, *D8Mit3*, *D8Mit4*, *D8Mit5*, *D8Mit6*, *D8Mit7* (defining the *ApocIII* gene), *D8Mit12*, *D8Rat2*, *D8Rat11*, *D8Rat20*, *D8Rat21*, *D8Rat24*, *D8Rat26*, *D8Rat34*, *D8Rat35*, *D8Rat36*, *D8Rat37*, *D8Rat38*, *D8Rat39*, *D8Rat40*, *D8Rat41*, *D8Rat42*, *D8Rat43*, *D8Rat44*, *D8Rat48*, *D8Rat49*, *D8Rat51*, *D8Rat53*, *D8Rat65*, *D8Rat71*, *D8Rat72*, *D8Rat75*, *D8Rat78*, *D8Rat 84*, *D8Rat85*, *D8Rat98*, *D8Rat103*, *D8Rat111*, *D8Rat113*, *D8Rat124*, *D8Rat127*, *D8Rat135*, *D8Rat143*, *D8Rat149*, *D8Rat150*, *D8Rat151*, *D8Rat152*, *D8Rat153*, *D8Rat154*, *D8Rat156*, *D8Rat157*, *D8Rat158*, *D8Rat164*, *D8Rat186*, *D8Rat198*, *D8Rat199*, *D8Rat 215*, *D8Rat216*, *D8Rat223*, *D8Rat227*, *D8Rat228*, *D8Wox6* (defining the *Rbp2* gene), *D9Bro1* (defining the solute carrier family 4, member 3, anion exchange protein gene), *D9Mit1*, *D9Mit2* (defining the crystallin γ polypeptide gene), *D9Mit4*, *D10Mgh7*, *D10Mit1* (defining the dipeptidyl carboxypeptidase 1 also designated as angiotensin I-converting enzyme, *Ace* gene), *D10Mit8* (defining the synaptobrevin 2 gene), *D10Rat61*, *D10Rat69*, *D10Rat116*, *D10Rat133*, *D10Rat157*, *D10Rat211*, *D10Rat240*, *Smst* (defining the somatostatin gene), *D11Mit1*, *D11Mit2*, *D11Mit4*, *D12Mit1*, *D12Mit2* (defining the plasminogen activator inhibitor gene), *D12Mit3*, *D12Mit4*, *D12Mit8*, *D13Mit2*, *D13Mit4*, *D13Mit5*, *D13Uwm1* (defining the renin gene), *D14Mit5*, *D14Mit7* (defining the albumin gene), *D14Mit9*, *D15Mit3*, *D16Mit2*, *D16Mit3*, *D16Mit5*, *D1A* (defining the dopamine-1A receptor gene), *D17Mit2*, *D17Mit4* (defining the cholinergic receptor, muscarinic 3, gene), *D18Mit2*, *D18Mit4*, *D18Mit8*, *D18Mit10*, *D19Mit2*, *Eta* (endothelin receptor type A gene), *R154*, *Tat* (tyrosine aminotransferase gene), *D20Mgh1*, *D20Mgh5*, *D20Mit1*, *D20Uwm1* (defining the tumor necrosis factor α gene), *DXMgh7*, *DXMit2* (myc-like oncogene, s-myc protein gene), *DXMit5*, *DXRat38*. In the case of chromosome 4 markers defining *Cd36/Fat* and *Pparγ*, PCR products were cleaved by restriction enzymes *Hinf*I and *Rsa*I, respectively. Further information about microsatellite markers and their polymorphisms among different rat strains can be found at: http://ratmap.gen.gu.se and http://www.resgen.com.

The characteristics of metabolic phenotypes

The experiments were performed on male adult rats 3–4 months of age. The rats had free access to water and standard chow. They were fed on a high sucrose diet (70% of energy sucrose) for two weeks before the end of the experiment. All experimental data were estimated under high sucrose diet conditions. The awake mean blood pressure was measured by the tail-cuff plethysmography.

The serum concentrations of triglycerides, free fatty acids and glucose were determined enzymatically using commercially available kits (Lachema, Brno, Czech Republic; Boehringer Mannheim, Germany). Serum insulin concentration was determined using the RIA kit for rat insulin assay (Amersham Pharmacia Biotech UK Ltd., Little Chalfont, UK). In the present study, insulin sensitivity of rat adipose tissue to insulin action was determined as described previously (Vrána and Kazdová, 1970). Insulin sensitivity was determined from the effect of insulin on the incorporation of ^{14}C-U glucose (Institute for Research and Application of Radioisotopes, Řež, Czech Republic) into total lipids *in vitro*. After decapitation, distal parts of the epididymal adipose tissue (200–230 mg) were incubated for 2 h in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 0.1 μCi/ml of ^{14}C-U glucose, 5 mmol/l of unlabeled glucose, and 2.5 mg/ml of bovine serum albumin (Fraction V; Armour Pharmaceuticals, Collegeville, PA), with or without insulin (250 μU/ml). The atmosphere was 95% O_2 + 5% CO_2. Total adipose tissue lipids were extracted according to Folch et al. (1957) and the radioactivity was determined as described previously (Vrána and Kazdová, 1970). Statistical analysis was performed by unpaired t-test for two-tailed P value.

Results

Microsatellite genotyping of PD/Cub, SHR and BN strains

The genotyping of the PD/Cub strain with more than 170 PCR microsatellite markers, defining either known genes (37) or mostly anonymous DNA sequences, confirmed the highly inbred status of this strain. No case of residual heterozygosity was found in this analysis. From the total number of 176 microsatellite markers,

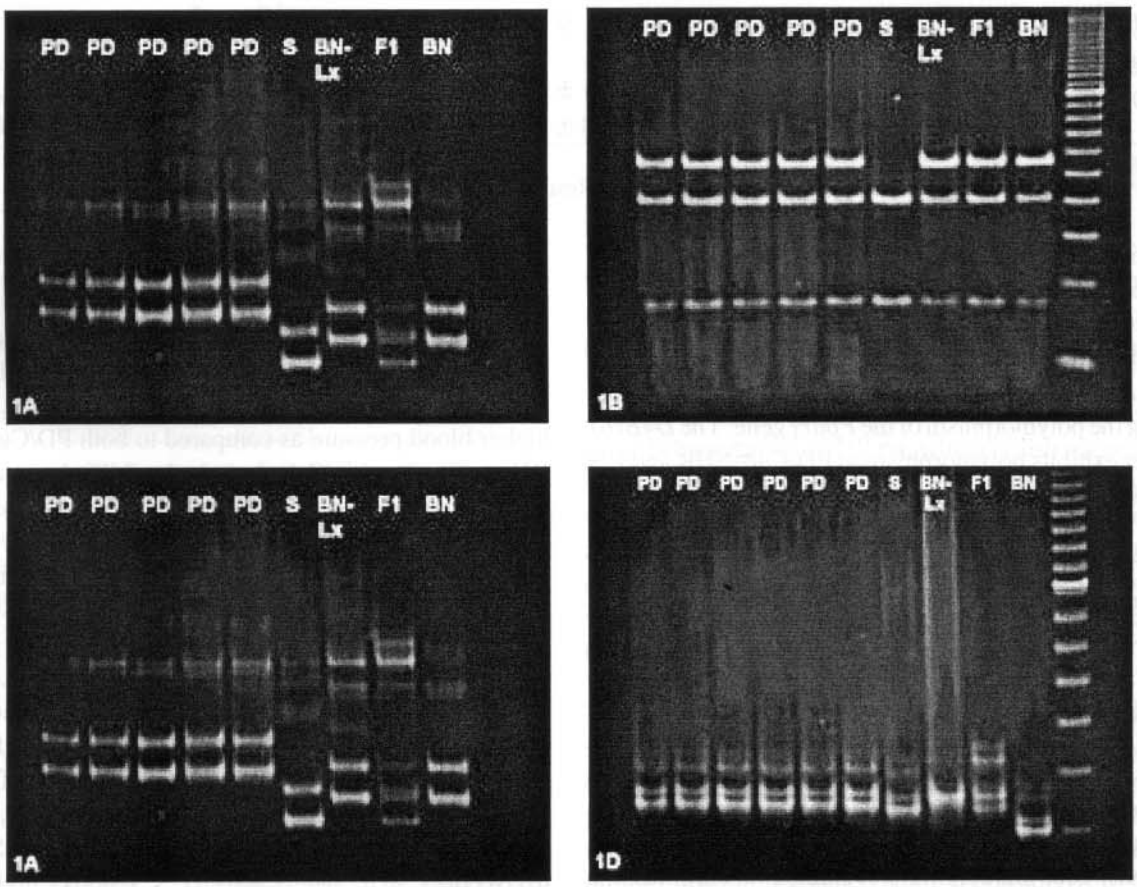

Fig. 1. Polymorphisms in PCR products among PD/Cub (PD), SHR (S), BN-*Lx* (congenic strain which carries the differential segment of chromosome 8 of PD origin including the *ApocIII* gene), F1(SHRxBN-*Lx*) and BN strains for different microsatellite markers. Size markers used: low ladder (Sigma) for *Cd36/Fat* and *ApocIII*, and pBRHaeIII digest for *Pparγ*. Fig. 1A for *D4Bro1* (defining *Slc4a2*), 1B for *Cd36/Fat*, 1C for *Pparγ*, 1D for *ApocIII*. In Fig. 1C, the abbreviation Sn designates uncleaved SHR PCR product and PDn designates uncleaved PD PCR product.