Differential Linkage of Triglyceride and Glucose Levels on Rat Chromosome 4 in Two Segregating Rat Populations

(insulin resistance syndrome / PD/Cub / BN.SHR4 / congenic strain / QTL / Cd36/FAT)

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Abstract. The PD/Cub is a recently established model of the IRS. The BN.SHR4 congenic strain was derived by introgression of the chromosome 4 segment of SHR origin (including the defective *Cd36/Fat* allele) onto the BN/Cub genetic background. We investigated the linkage of metabolic and morphometric phenotypes (total body weight, OGTT, fasting serum levels of TG, FFA) on chromosome 4 in two separate F2 rat populations: the PD/Cub x BN/Cub and PD/Cub x BN.SHR4 (total N = 243). In the PD/Cub x BN.SHR4 F2s, we found significant linkage for fasting TG levels (LOD = 3.26) and suggestive linkage for fasting glycaemia (LOD = 2.80) in the interval *Il-6 – D4Bro1*, i.e. the part of chromosome 4 of SHR origin in the BN.SHR4 congenic. However, no linkage for fasting TG concentrations, fasting glycaemia or any other followed parameter was found in the second, PD/Cub x BN/Cub F2. The differential linkage of TG and glucose levels to the centromeric part of rat chromosome 4q in the studied F2s points to the importance of this region for the lipid and carbohydrate metabolism at the specific age (10 months) and diet (standard chow) combination. The *Cd36/Fat* and *Il-6* genes are the preliminary positional candidates for the observed effect.

Metabolic syndrome (or insulin resistance syndrome, IRS) represents a clustering of metabolic and haemodynamic dysregulations often found in a single patient (Reaven, 1988). The prevalence of this disease is reaching a startling rate both in affluent and developing countries (Meigs et al., 2003), eliciting an urgent need for proper understanding of the causative mechanisms and subsequent determination of effective treatment modalities. Though its causes remain to be elucidated, the IRS seems to result from multiple interacting factors with a documented strong role of the genetic component. Adding to the complexity of its pathogenesis, numerous environmental variables (e.g. diet, stress, medication) interact with the genetic component of IRS. The possibilities offered by use of animal models for the study of complex diseases are particularly pertinent in such an intricate setting. By using genetically defined animal strains in a specified set of combinations of environmental conditions at several ontogenetic stages, it may be feasible to decipher not only single gene variants associated with IRS, but also "protective" and "predisposing" states such as combinations of age, nutritional status and genetic makeup (allelic variants at several loci or haplotype combinations).

The polydactylous rat strain (PD/Cub) is a highly inbred rat strain kept since 1969 at the Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University in Prague. It carries a mutant allele of the *Lx* gene, which gives rise to the polydactyly-luxate syndrome (Křen, 1975). The PD/Cub rat strain (together with congenic and recombinant inbred strains derived from it) has been exploited as a model of limb development and

Received October 16, 2003. Accepted November 19, 2003.

This work was supported by following grants: No. 303/01/1010 and No. 204/98/K015 from the Grant Agency of the Czech Republic and 6367-3 from the Internal Grant Agency of the Ministry of Health of the Czech Republic, CIHR MT-14654, GEI-53958. O.Š. is a recipient of TACTICS fellowship.

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Abbreviations: ANOVA – analysis of variance, AUC – area under curve, Cd36/Fat – fatty acid translocase, cM – centiMorgan, FFA – free fatty acids, IRS – insulin resistance syndrome, OGTT – oral glucose tolerance test, QTL – quantitative trait locus, RNO – rat chromosome, TG – triglyceride.

teratology (Křen et al., 1996; Bílá et al., 2000), hypertriglyceridaemia (Vrána et al., 1993) and it was established as a model for IRS (Šedová et al., 2000). Recently, the PD/Cub rat was shown to possess a distinct pharmacogenetic profile in response to thiazolidinediones (Šeda et al., 2002a), fibrates and retinoids (Šedová et al., personal communication). 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). Brown Norway rat was transferred from the USA to the Institute of Biology, First Faculty of Medicine 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 normotriglyceridaemic. The BN.SHR-4(*Il6/Cd36)* or BN.SHR4 congenic strain was derived by introgressing the RNO4 differential segment of SHR origin onto the BN genetic background (Šeda et al., 2002b). The BN.SHR4 displays several derangements of carbohydrate and lipid metabolism and the differential segment was shown to be involved in pharmacogenetic interaction with rosiglitazone (Šeda et al., 2003a), blunting its action in this strain.

In this study, we analysed the linkage of metabolic and morphometric phenotypes on chromosome 4 in two separate F2 rat populations: the PD/Cub x BN/Cub and PD/Cub x BN.SHR4. The selection of this chromosome in particular was driven by previous findings suggesting a substantial importance of this region (especially the *Cd36/Fat* gene) for the metabolic syndrome-related derangements (Aitman et al., 1999; Pravenec et al., 2001). Moreover, the RNO4 of the two separate F2 progenies combines either BN/Cub and PD/Cub alleles or SHR (including the defective *Cd36/Fat*) and PD/Cub, allowing us to test the relevance of each set by differential linkage.

Material and Methods

Rat F2 intercross populations

By reciprocally crossing PD/Cub and BN/Cub progenitors, the F1 hybrids (PD/Cub x BN/Cub) were obtained. These were further crossed to generate F2 hybrids. Only male F2 rats were used in the study (N = 149). Similarly, the PD/Cub x BN.SHR4 F2 population was derived (N = 94).

Metabolic measurements

The rats had free access to water and were fed standard rodent chow. Male F2 rats (10 months of age, total N = 243) and the progenitors – PD/Cub (N = 6), BN/Cub (N = 5) and BN.SHR4/Cub (N = 6) were weighed and the serum concentrations of triglycerides (TG), free fatty acids (FFA), insulin and glucose were determined as described previously (Šedová et al., 2000) in fasted animals. The oral glucose tolerance test (OGTT) was performed after overnight fasting. Blood for glycaemia determination was drawn from the tail at intervals of 0, 30, 60 and 120 min after the intragastric glucose administration to conscious rats (3 g/kg total body weight, 30% aqueous solution).

Genotyping and linkage analysis

Microsatellite markers *Il-6, D4Bro1, D4Rat7, D4Mgh22, D4Rat115, D4Rat9, D4Rat196, D4Rat76, D4Rat141, D4Wox16, D4Rat68, D4Rat69* and *D4Rat206* were used for genotyping. We performed the interval mapping on the dataset of obtained phenotype and genotype information using the MapManager programme (v. 0.24; Manly et al., 2001).

Statistical analysis

The normality of distribution of the measured phenotypes was assessed using the Kolmogorov-Smirnov test. When positive, the values were normalized using a simple function transformation (log, square root) and the normalized values were used for interval mapping analyses. When comparing more than two groups, one-way ANOVA was used with the post-hoc Tukey's honest significance difference test for comparison of the specific pairs of variables. For comparisons of only two groups, Student's t-test was used. Null hypothesis was rejected whenever $P < 0.05$.

Results and Discussion

Metabolic profile of the two F2 populations and their progenitors

The metabolic profile of PD/CubxBN/Cub and PD/CubxBN.SHR4 F2 populations are shown in Table 1 and Table 2, respectively. Overall, the F2 rats showed values between those observed in progenitor strains; however, mostly much closer to those of BN/Cub or BN.SHR4, respectively, indicating a recessive nature of PD/Cub alleles responsible for the observed pathological phenotypes. Interestingly, the two segregating populations differed significantly in the distributions of the trait values, fasting glycaemia ($P < 0.001$) and area under the curve (AUC) of OGTT ($P = 0.04$) being higher in PD/Cub x BN/Cub, while the levels of fasting insulin ($P = 0.01$) and FFA ($P < 0.001$) were higher in PD/Cub x BN.SHR4 F2. These disparities might have been caused by the SHR alleles present in the differential segment of RNO4 in the BN.SHR4 strain, as this genomic region solely differentiates the genomic pool of the two F2 populations. In order to assess whether the genes present on RNO4 are affecting the followed traits, linkage analysis was performed.

Linkage analysis

In the PD/Cub x BN.SHR4 F2s, we found significant linkage for fasting TG levels (LOD = 3.26) and suggestive linkage for fasting glycaemia (LOD = 2.80) in the interval *Il-6 – D4Bro1*, i.e. the part of chromosome 4 (RNO4) of SHR origin in the BN.SHR4 congenic (Fig. 1). However, no linkage for fasting TG concentrations,

Table 1. Metabolic profile of the PD/CubxBN/Cub F2 population and its progenitors

	BN/Cub (N = 5)		PD/CubxBN/Cub F2 (N = 149)				PD/Cub (N = 6)	
	Mean	**SEM**	**Mean**	**Min**	**Max**	**SEM**	**Mean**	**SEM**
Total body weight (g)	**274.1**	10.27	**329.89**	233.2	448.5	3.75	**376.9**	3.32
Glucose (mmol/l)	**4.28**	0.09	**4.43**	3.3	6.1	0.05	**5.24**	0.19
AUC (OGTT; mmol/l/2 hod)	**795**	16.66	**767.08**	591	1234.5	7.24	**833.4**	17.97
Triglyceride (mmol/l)	**0.54**	0.06	**0.52**	0.23	1.4	0.02	**2.02**	0.24
Insulin (pmol/l)	**0.11**	0.02	**0.15**	0.07	1.15	0.01	**0.23**	0.01
Free fatty acids (mmol/l)	**1.12**	0.09	**0.88**	0.32	1.47	0.02	**1.05**	0.05

SEM - standard error of mean, Min - minimal value for a given phenotype found in F2, Max - maximal value for a given phenotype found in F2, AUC (OGTT) - area under the curve of the oral glucose tolerance test

Table 2. Metabolic profile of the PD/CubxBN.SHR4 F2 population and its progenitors

	BN.SHR4 (N = 6)		PDxBN.SHR4 (N = 94)				PD/Cub (N = 6)	
	Mean	**SEM**	**Mean**	**Min**	**Max**	**SEM**	**Mean**	**SEM**
Total body weight (g)	**257**	8.9	**336.18**	223.3	451.7	4.61	**376.9**	3.32
Glucose (mmol/l)	**4.7**	0.18	**4.19**	3.3	5.6	0.05	**5.24**	0.19
AUC (OGTT; mmol/l/2 hod)	**702.8**	25.3	**744.09**	579	994.5	8.21	**833.4**	17.97
Triglyceride (mmol/l)	**1.24**	0.02	**0.55**	0.28	1.2	0.02	**2.02**	0.24
Insulin (pmol/l)	**0.14**	0.02	**0.18**	0.08	0.485	0.01	**0.23**	0.01
Free fatty acids (mmol/l)	**1.1**	0.36	**1.37**	0.6	5.38	0.08	**1.05**	0.05

SEM - standard error of mean, Min - minimal value for a given phenotype found in F2, Max - maximal value for a given phenotype found in F2, AUC (OGTT) - area under the curve of the oral glucose tolerance test

fasting glycaemia or any other measured parameter was found in the second, PD/Cub x BN/Cub F2. When comparing the three genotype groups within the PD/Cub x BN.SHR4 F2s, the BN.SHR4/BN.SHR4 homozygotes at the *D4Bro1* marker (the closest marker to the QTL peak) showed values of significantly lower glycaemia (3.88 ± 0.09 mmol/l) and TG (0.43 ± 0.02 mmol/l) when compared to BN.SHR4/PD heterozygotes (glycaemia 4.29 ± 0.06 mmol/l, P = 0.003; TG 0.57 ± 0.02 mmol/l, P = 0.004) or PD/PD homozygotes (glycaemia 4.27 ± 0.11 mmol/l, P = 0.01; TG 0.61 ± 0.04 mmol/l, P = 0.002). This finding is surprising, as it demonstrates lowering of TG and glucose levels in the presence of alleles of SHR origin, contrasting with previous reports concerning several QTLs linked to the RNO4 region in question. Apart from the original insulin resistance gene 1 (*Irg1*) QTL that led to the identification of the *Cd36/Fat* mutation in SHR (Aitman et al., 1999; Pravenec et al., 2001), the traits linked to this genomic region include mean arterial blood pressure (Pravenec et al., 1995), retroperitoneal fat weight in the OLETF rat (Ogino et al., 2000), HDL-2 phospholipids (Bottger et al., 1996) and TG in BB/OKx(SHR/MolxBB/OK) backcross (Kovacs and Kloting, 1998). In the latter, the SHR alleles increased TG, as was the case in the BN.SHR4 congenic rat strain (Šeda et al., 2002b) and *Cd36*-null mice (Hajri et al., 2002). It must be kept in mind that in spite of the clearly present linkage to the *Cd36/Fat*-containing region of RNO4, a) there are several genes other than *Cd36/Fat* present within the genomic interval of the peak linkage, such as interleukin 6; b) both triglyceridaemia and glycaemia are probably affected by more than one locus (Šeda et al., 2003b,c); therefore, interactions with other loci may play a role in determination of the observed values. Such interaction of the discussed RNO4 segment with genetic background is apparent e.g. in different response of the SHR/OlaIpcv and BN.SHR4/Cub strains to thiazolidinediones (Qi et al., 2002; Šeda et al., 2003a). Also, the BN-*Lx* 1K triple congenic strain, in which a relatively small region of RNO4 containing the *Cd36/Fat* gene of SHR origin is present on the BN/Cub genetic background together with the RNO20 segment of SHR origin and RNO8 segment of PD/Cub origin, displays substantially more pronounced dyslipidaemia and glucose intolerance than that found in the BN.SHR4/Cub or BN-*Lx* congenic strains (Šeda et al., 2002b). Nevertheless, *Cd36/Fat* may be considered a candidate gene responsible for the lower glucose concentrations at the standard diet conditions, as, for example, the *Cd36*-deficient mice were reported to be hypoglycaemic and only after a dietary challenge was the glucose tolerance impaired (Hajri et al., 2002). In summary, we report a case of differential linkage of TG and glucose levels to the RNO4 interval *Il-6 – D4Bro1* in two segregating rat populations involving a model of the insulin resistance syndrome, polydactylous rat strain. The presented results contribute to the elucidation of the role of this genomic region in deranged lipid and carbohydrate metabolism at distinct ontogenetic and gene-environmental settings.

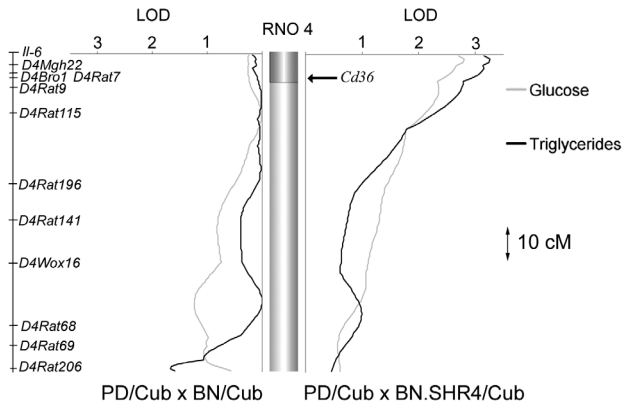

Fig. 1. Differential linkage of triglyceride and glucose levels on rat chromosome 4 in PD/CubxBN//Cub (left) and PD/CubxBN.SHR4 (right) F2 populations. The dark segment of chromosome 4 indicates the region of SHR origin in the BN.SHR4 congenic strain, the position of *Cd36/Fat* is indicated by the arrow. The genotyped markers are shown (far left) at distances computed by the MapManager QTX programme (v .0.24; Manly et al., 2001)

Acknowledgments

We thank Ms. Blanka Chylíková, Helena Habrmanová, Michaela Janků, Zdeňka Kopecká and Marie Uxová for their technical assistance.

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