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RESEARCH LETTER

Gene expression profiling supports the role of *Repin1* in the pathophysiology of metabolic syndrome

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Abstract Congenic BB rat strains carrying a SHR segment (D4Got41-Tacr1; 60.5–122.8 Mb; BB.4S) or a WOKW segment (D4Got41-Fabp1; 60.5–104.6 Mb; BB.4W) of chromosome 4 within the BB/OK background develop facets of the metabolic syndrome when compared with their parental BB/OK rats. To narrow down potential genes involved in the pathophysiology of metabolic syndrome, gene expression studies in adipose tissues of BB/OK, BB.4S, and BB.4W rats were initiated. Total RNA of subcutaneous and epididymal adipose tissue of BB/OK ($n = 10$), congenic BB.4S ($n = 8$), and BB.4W ($n = 9$) males at an age of 4 weeks was isolated. The mRNA expression of 92 genes involved in obesity, insulin resistance and other metabolic traits was measured by RT-PCR. Significant differences in gene expression were only found in *Repin1* in both adipose tissues. Congenic BB.4W showed significantly lower gene expression than did BB.4S and BB/OK. Our findings and newly published findings of *Repin1* in 3T3-L1 adipocytes support the hypothesis that *Repin1* may affect the development of facets of the metabolic syndrome.

Keywords Obesity · Metabolic syndrome · Congenic rats

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Introduction

Studies in humans have shown clustering of hyperinsulinemia, glucose intolerance, dyslipidemia, hypertension, and obesity, which has been referred to as the metabolic syndrome [1]. The metabolic syndrome is a polygenetically inherited disease that complicates studies in humans. Several animal models developing aspects of metabolic syndrome are used to study the interaction of genetic and environmental factors. For instance, the spontaneously hypertensive rats (SHR) develop obesity, insulin resistance, hypertriglyceridemia, and hypertension and the Wistar Ottawa Karlsburg RT1^{u} (WOKW) rats develop obesity, dyslipidemia, impaired glucose tolerance, hyperinsulinemia, and hyperleptinemia [2, 3].

In several crossing studies quantitative trait loci (QTLs) were found for serum triglycerides and total cholesterol mapped on chromosome 4 [4–6]. To determine the physiologic relevance of these QTLs, two congenic BB rat strains were established using SHR or WOKW rats as donors of chromosome 4 segment. The phenotypic characterization of both congenic strains termed BB.4S (D4Got41-Tracr1; 60.5–122.8 Mb) and BB.4W (D4Got41-Fabp1; 60.5–104.6 Mb) showed that the strains develop obesity, dyslipidemia, hyperleptinemia (BB.4S) or hyperinsulinemia (BB.4W) compared with parental strain BioBreeding/OttawaKarlsburg (BB/OK). In contrast to parental BB/OK rats developing type 1 diabetes at a frequency of more than 86% [7], none of the congenic BB.4S [8], and BB.4W [6] rats developed diabetes. All congenic rats remained normoglycemic up to an age of 30 weeks, which was attributable to the lack of diabetogenic gene *Iddm2* (*Gimap5*), an essential gene for type 1 diabetes development in BB/OK rats.

Adipose tissue is currently considered as a hormonally active system in the control of metabolism [9]. This tissue

secretes a large number of physiologically active peptides which are changed through obesity and may influence development of metabolic syndrome [10]. To narrow down potential genes underlying facets of the metabolic syndrome in these congenic strains, gene expression analysis was carried out for 92 genes located on chromosome 4 or genes known to be involved in obesity, insulin resistance, and other facets of metabolic syndrome, indicating possible gene interactions [11]. In this study, the gene expression in epididymal and subcutaneous adipose tissue in both congenic strains (BB.4S, BB.4W) and rats of their parental strain BB/OK was examined at an age of 4 weeks because potential candidate genes will show differences at any age.

Materials and methods

All rats used were bred and kept in our own animal facility in Macrolon type III cages (Ehret GmbH, Emmendingen, Germany) under strict hygienic conditions. They were maintained in a 12-h light/dark cycle (5:00 AM/5:00 PM). All rats were free of major pathogens and had free access to food (Ssniff; Soest, Germany) and acidulated water. The experiments were performed in accordance with the rules for animal care of the Ministry of Nutrition, Agriculture and Forestry of the German Government.

All rats were killed at an age of 4 weeks by an overdose of anesthetic (Sevofluran, Abbott). Epididymal and subcutaneous adipose tissue was removed. Total RNA of subcutaneous and epididymal adipose tissue of BB.OK ($n = 10$), BB.4S ($n = 8$), and BB.4W ($n = 9$) males was isolated, transcribed in cDNA, and used for real-time PCR as described previously [12]. Target cDNA was amplified by primer sets of 92 genes (Table 1) involved in obesity, insulin resistance and other metabolic traits. For each experimental sample, the amounts of targets and endogenous reference (18S rRNA) were determined from the calibration curve.

Data are given as mean ± SD. Statistical significance was assessed using R 2.10.0 a free software environment for statistical computing and graphics. The Wilcoxon rank sum test was used to test the equality of the means of the two examples. Multiple tests were performed by Bonferroni corrections. Differences were considered to be statistically significant at a level of $P < 0.05$.

Results

As shown in Table 1, 44 out of 92 (third column) and 88 out of 92 (sixth column) genes were expressed in epididymal and subcutaneous adipose tissue, respectively. 40 of 92 genes were expressed in both tissues. Only 1 out of 92 genes showed a significantly different relative gene expression between BB.4S and BB.4W rats in both tissues. This difference in gene expression was found in *Repin1* in epididymal ($P = 0.004$) and subcutaneous ($P = 0.007$) adipose tissue. The statistical analysis showed clearly that *Repin1* on chromosome 4 is significantly more highly expressed in BB.4S compared to BB.4W rats (Fig. 1). In contrast to their parental strain BB/OK, congenic BB.4S did not show significantly higher gene expression of *Repin1*.

Discussion

In contrast to their parental strain BB/OK, congenic BB.4S and BB.4W do not develop type 1 diabetes, but they manifest facets of metabolic syndrome [6]. Because BB.4S and BB.4W showed comparable facets of metabolic syndrome, it was not surprising that both congenic strains differed in the relative expression of selected genes when compared with their parental BB/OK strain. However, the number of differing genes was very low. Only 1 out of 92 genes showed significantly different mRNA expression between the two congenics. *Repin1* is mapped on chromosome 4q24 and is, therefore, located in the introgressed chromosomal region of both congenic strains BB.4S and BB.4W.

The decreased mRNA levels of *Repin1* in adipose tissue of BB.4W in relation to BB.4S and parental strain BB/OK could partially explain the phenotype of WOKW as a chromosomal donor of BB.4W. As previously reported we have identified triplet repeats (TTT) in the 3′-untranslated region (UTR) of *Repin1* that correlated with facets of the metabolic syndrome [12]. The sequencing of the *Repin1* triplet T repeat showed that spontaneously diabetes-prone BB rats possess 9 and the chromosomal donors SHR and WOKW rats possess 5 and 11 repeats, respectively. Since variation of the AT content in the 3′-UTR could influence the gene expression [13], one might speculate that the low number of SHR repeats could cause the higher gene expression of *Repin1* in BB.4S rats compared to BB.4W, which carries the highest number of T repeats.

In another study, Ruschke et al. [14] demonstrated that *Repin1* regulates the expression of genes involved in adipogenesis, lipid droplet formation and fusion, and glucose and fatty acid transport in 3T3-L1 adipocytes. Furthermore, *Repin1* knockdown causes a significant reduction of glucose transporters 1 (GLUT1) and a significant increase of glucose transporters 4 (GLUT4) at the protein level which are involved in basal glucose uptake and insulin-stimulated glucose uptake, respectively. These findings indicate that these glucose transporters are most probably *Repin1* target genes. However, the relative gene expression of *Glut4* was

Table 1 List of genes studied

Genes	Chromosome	Epididymal adipose tissue	*P* value	Bonferroni adjustment	Subcutaneous adipose tissue	*P* value	Bonferroni adjustment
Cnksr3	1p11				x	>0.05	
Cebpa	1q21				x	>0.05	
Sv2b	1q31				x	>0.05	
Mki67_1	1q41	x	>0.05		x	>0.05	
Mki67_2	1q41	x	>0.05		x	>0.05	
Foxo1	2q26	x	>0.05		x	>0.05	
Lcn2	3p11	x	>0.05		x	>0.05	
Il1b	3q36	x	**0.0208**	>0.05	x	>0.05	
Cebpb	3q42				x	>0.05	
Il6	4q11				x	>0.05	
Sema3a	4q12				x	>0.05	
Lep	4q22	x	>0.05		x	>0.05	
Nrf1	4q22				x	>0.05	
Plxna4	4q22				x	>0.05	
Smo	4q22				x	>0.05	
Ephb6	4q22				x	>0.05	
Epha1	4q23				x	>0.05	
Zyx	4q23				x	>0.05	
Npy	4q24				x	>0.05	
Nrf3	4q24				x	>0.05	
Snca	4q24	x	>0.05		x	>0.05	
Snx10	4q24	x	>0.05		x	>0.05	
Aqp1	4q24				x	>0.05	
Osbpl3	4q24				x	>0.05	
Repin1	4q24	x	**0.00008**	**0.004**	x	**0.00008**	**0.007**
Ian4	4q24	x	>0.05		x	**0.0079**	>0.05
Pparg	4q42	x	**0.0303**	>0.05	x	>0.05	
Tgfbr1	5q22	x	>0.05		x	>0.05	
Lepr	5q33	x	>0.05		x	>0.05	
Cdc42	5q36				x	>0.05	
Visfatin	6q16	x	>0.05		x	>0.05	
Gphn	6q24				x	>0.05	
Sos2	6q24				x	>0.05	
Tgfb3	6q31	x	>0.05		x	>0.05	
Tgfb1	6q31	x	>0.05		x	>0.05	
Cdc42bpb	6q32	x	>0.05		x	>0.05	
Dlk1	6q32	x	**0.0037**	>0.05	x	>0.05	
Yy1	6q32	x	>0.05		x	>0.05	
Begain	6q32	x	**0.0081**	>0.05	x	>0.05	
Akt	6q32	x	>0.05		x	>0.05	
Sno1	6q32	x	>0.05		x	>0.05	
Gtl2	6q32	x	>0.05		x	**0.0111**	>0.05
WD40	6q32	x	>0.05		x	>0.05	
Yy-Micr	6q32				x	>0.05	
Yy-Micr	6q32				x	>0.05	
miYy1	6q32	x	>0.05				
Dicer	6q32	x	>0.05				

Table 1 continued

Genes	Chromosome	Epididymal adipose tissue	*P* value	Bonferroni adjustment	Subcutaneous adipose tissue	*P* value	Bonferroni adjustment
Rtl1rev	6q32	x	>0.05				
Rtl1	6q32	x	>0.05		x	**0.0464**	>0.05
Serpina12	6q32	x	>0.05		x	>0.05	
Micro	6q32	x	>0.05		x	>0.05	
Tgfbr2	8q32	x	>0.05		x	**0.0464**	>0.05
C3	9q11				x	>0.05	
Vegfa	9q12				x	>0.05	
Srebf1	10q22	x	>0.05		x	>0.05	
Glut4	10q24	x	>0.05		x	>0.05	
Ccl2	10q26	x	>0.05		x	>0.05	
Adipog	11q23				x	>0.05	
Serpine1	12q11-q12				x	>0.05	
Resistin	12q12				x	>0.05	
Limk1	12q12	x	>0.05				
p47phox	12q12	x	>0.05		x	>0.05	
Il10	13q13				x	>0.05	
Slam	13q24				x	>0.05	
Tgfbr3	14q22	x	>0.05		x	>0.05	
Npyr1	16p14				x	>0.05	
Ptch1	17p14				x	>0.05	
Neurog1	17p14				x	>0.05	
Ucp	19p11-q11				x	>0.05	
Nrp1	19q12				x	>0.05	
Tnfa	20p12				x	>0.05	
Mif	20p12	x	>0.05		x	>0.05	
Hsp70_1	20p12				x	>0.05	
Hsp70_2	20p12				x	>0.05	
Hsp70_3	20p12				x	>0.05	
Syngap1	20p12				x	>0.05	
Gria3	Xq11	x	>0.05		x	>0.05	
Birc4	Xq11				x	>0.05	
Rgn	Xq11-q12				x	>0.05	
Maob	Xq12				x	>0.05	
Maoa	Xq12				x	>0.05	
Syn1	Xq12	x	>0.05		x	>0.05	
Cask	Xq12				x	>0.05	
Np15.6	Xq12	x	>0.05		x	**0.0152**	>0.05
Foxp3	Xq13				x	>0.05	
Gripap1	Xq13				x	>0.05	
Bmp15	Xq13	x	>0.05		x	**0.0274**	>0.05
Pak3	Xq14				x	>0.05	
Ar	Xq22-q32	x	>0.05		x	>0.05	
Efnb1	Xq22.1				x	>0.05	
Apelin	Xq35				x	>0.05	
Plxna3	Xq37	x	>0.05		x	>0.05	

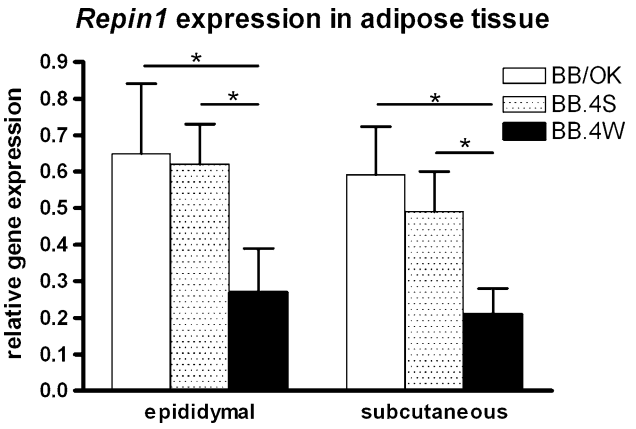

Fig. 1 Significantly different gene expression of *Repin1* (locus 4q24) in subcutaneous and epididymal adipose tissue of BB/OK, BB.4S and BB.4W ($*P < 0.01$)

not significantly different between BB.4S and BB.4W rats. However, this finding may explained by the fact that the gene expression was measured and not the protein expression as determined in 3T3-L1 adipocytes [14].

The differences found in *Repin1* gene expression between the two congenics on the one hand and between BB/OK, BB.4S, and BB.4W on the other may be an indication that *Repin1* plays causal role in adipose tissue underlying facets of the metabolic syndrome.

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Conflicts of interest The authors have no conflicts to disclose.

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