Physiol. Res. 52: 681-688, 2003

Transgenic Expression of CD36 in the Spontaneously Hypertensive Rat Is Associated with Amelioration of Metabolic Disturbances But Has No Effect on Hypertension

M. PRAVENEC[1,2], V. LANDA[1], V. ZÍDEK[1], A. MUSILOVÁ[1], L. KAZDOVÁ[3], N. QI[4], J. WANG[4], E. ST. LEZIN[4,5], T. W. KURTZ[4]

[1]Institute of Physiology, Czech Academy of Sciences and Center for Integrated Genomics, [2]Institute of Biology and Medical Genetics, First Medical Faculty, Charles University, [3]Institute for Clinical and Experimental Medicine, Prague, Czech Republic, [4]Department of Laboratory Medicine, University of California and [5]Department of Veterans Affairs Medical Center, San Francisco, CA, USA

Received August 29, 2002
Accepted November 25, 2002

Summary

Spontaneously hypertensive rats (SHR/NIH strain) harbor a deletion variant in the *Cd36* fatty acid transporter and display defective fatty acid metabolism, insulin resistance and hypertension. Transgenic rescue of *Cd36* in SHR ameliorates insulin resistance and improves dyslipidemia. However, the role of *Cd36* in blood pressure regulation remains controversial due to inconsistent blood pressure effects that were observed with transgenic expression of *Cd36* on the SHR background. In the current studies, we developed two new SHR transgenic lines, which express wild type *Cd36* under the control of the universal Ef-1α promoter, and examined the effects of transgenic expression of wild type *Cd36* on selected metabolic and cardiovascular phenotypes. Transgenic expression of *Cd36* in the new lines was associated with significantly decreased serum fatty acids, amelioration of insulin resistance and glucose intolerance but failed to induce any consistent changes in blood pressure as measured by radiotelemetry. The current findings confirm the genetic association of defective *Cd36* with disordered insulin action and fatty acid metabolism in the SHR/NIH strain and suggest that *Cd36* is linked to other gene(s) on rat chromosome 4 that regulate blood pressure.

Key words

SHR • *Cd36* • Transgenic animal • Blood pressure • Insulin resistance • Fatty acids

Introduction

Patients with the hypertension and metabolic syndrome typically develop several abnormalities of lipid and carbohydrate metabolism which are often accompanied by increased blood pressure, visceral obesity as well as defects in coagulation (Reaven 1993, 1995, Groop and Ortho-Melander 2001). Epidemiological analyses (Neel *et al*. 1998, Pedersen 1999, Beck-Nielsen *et al.* 1999, Groop 2000, Jensen 2000, Ukkola and Bouchard 2001) and studies in animal models (Ernsberger *et al*. 1999, Kovacs *et al*. 2000, Mauvais-

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

Jarvis and Kahn 2000, Kadowaki 2000, Šedová *et al*. 2000, Štolba *et al*. 1992) have indicated that this cardiovascular risk factor clustering is under significant genetic control. However, the molecular lesions responsible for these defects remain unknown.

The spontaneously hypertensive rat (SHR) is the most widely used animal model of human essential hypertension which under appropriate dietary conditions exhibits multiple metabolic disorders similar to the human dysmetabolic syndrome (Aitman *et al*. 1997, Pravenec *et al*. 1999, 2000). Recently, a deletion variant in *Cd36*, which codes for a long-chain fatty acid transporter on cell membranes, was detected in the SHR/NIH strain and was linked to the transmission of insulin resistance and disordered fatty acid metabolism (Aitman *et al*. 1999). Transgenic expression of wild type *Cd36* under the control of a universal promoter in SHR harboring mutant *Cd36* ameliorated insulin resistance and lowered serum levels of fatty acids (Pravenec *et al*. 2001a). However, the effects of the wild type *Cd36* transgene expression on blood pressure regulation remained uncertain because the transgenic expression was associated with a modest amelioration of hypertension in only one of two transgenic lines (Pravenec *et al*. 2001b). This inconsistency raised the question of whether the change in blood pressure observed in the single transgenic line might have been related to the site of insertion of the transgene or to ectopic expression of the *Cd36* transgene rather than to correction of CD36 deficiency *per se*. In the current studies, we derived two additional SHR *Cd36* transgenic lines to further examine the role of *Cd36* in carbohydrate and fatty acid metabolism and its possible effect on blood pressure regulation.

Methods

Animals

We derived two new transgenic SHR lines, SHR/Ola-TgN(EF1aCd36)93Ipcv (abbreviated as SHR-TG93) and SHR/Ola-TgN(EF1aCd36)106Ipcv (abbreviated as SHR-TG106), by microinjecting SHR/Ola zygotes with wild type *Cd36* cDNA as described previously (Pravenec *et al*. 2001a). The *Cd36* cDNA construct was prepared from fat tissue of a Wistar -Kyoto (WKY) rat by RT-PCR using primers (upstream nucleotides 21-40; downstream nucleotides 1897-1916) designed from the GenBank accession number L19658. The cDNA was cloned into the Invitrogen TA cloning vector pCR2.1 and then subcloned into the Invitrogen vector pEF1/V5-HisA using *Bam*HI and *Xba*I restriction sites. The cloned cDNA was verified by direct sequencing. The construct was linearized by digesting the vector with *Aat*II and *Sma*I to isolate a 4.8 kb fragment for transgene injection that contained the Ef-1α promoter, the cDNA for *Cd36*, and a bovine growth hormone polyadenylation sequence. The recipient SHR/Ola strain descends from the SHR/NIH strain and was one of the original strains used to map phenotypes linked to *Cd36* deficiency (Aitman *et al*. 1999). Littermates not inheriting the transgene were used as negative controls in phenotyping studies.

Experimental protocol

From weaning, the rats were fed a standard chow, and at 8 weeks of age, the rats were fed a semi-synthetic diet containing 60% fructose (Hope Farms, The Netherlands) for 15 days. Serum glucose, insulin, triglycerides, and non-esterified fatty acids (NEFA) were measured in non-fasted male rats after 15 days of fructose feeding. On day 13 of the high fructose diet feeding, an oral glucose tolerance test (OGTT) was performed using a glucose load of 300 mg/100 g body weight after 7-hour fasting. Blood was withdrawn from the tail without anesthesia before the glucose load and then after 30, 60 and 120 min. Blood samples were clotted at 4 °C, centrifuged, and the sera were kept frozen until analysis.

All experiments were performed in agreement with the Animal Protection Law of the Czech Republic (311/1997) which corresponds fully to the European Community Council recommendations for the use of laboratory animals 86/609/ECC. The experiments were approved by the Ethics Committee of the Institute of Physiology, Czech Academy of Sciences, Prague.

Biochemical analysis

Blood glucose levels were measured by the glucose oxidase assay (Lachema, Brno, Czech Republic) using tail vein blood drawn into 5 % trichloracetic acid and promptly centrifuged. Serum NEFA levels were determined using an acyl-CoA oxidase-based colorimetric kit (Roche Diagnostics GmbH, Mannheim, Germany). Serum triglycerides and total cholesterol concentrations were measured by standard enzymatic methods (Lachema, Brno, Czech Republic). Serum insulin concentrations were determined using a rat insulin radioimmunoassay kit (Amersham Pharmacia Biotech, UK).

In vitro insulin sensitivity was determined in isolated diaphragmatic muscles by measuring the effect of insulin on incorporation of ^{14}C-U glucose into total glycogen and also in epididymal fat pad by measuring the effect of insulin on incorporation of ^{14}C-U glucose into fat. After decapitation, the diaphragmatic muscles or the epididymal fat pads 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 (Armour, Fraction V), without or with 250 μU/ml insulin. The tissues were incubated at 95 % O_2 + 5 % CO_2. Total glycogen from the diaphragmatic muscles and total lipids from the epididymal fat pads were extracted as described before (Vrána and Kazdová 1970).

Hemodynamic studies

Arterial blood pressures and heart rates were measured continuously in unanesthetized, unrestrained male SHR-TG93 and SHR-TG106 transgenic rats and their SHR-TG control littermates between 10 and 14 weeks of age using radiotelemetry. All rats were allowed to recover for at least 10 days after implantation of radiotelemetry transducers before the blood pressure recordings were started. Pulsatile pressures and heart rates were recorded in 5-second bursts every 5 min throughout the day and night for two weeks. 24-hour averages for mean arterial blood pressure and heart rate were calculated in each rat for each day of the study. The results from each rat in the same group were then averaged to obtained the group means. Rats were fed standard lab chow and tap water *ad libitum* throughout the study.

Statistical analysis

All data are expressed as means ± S.E.M. Average mean blood pressures and heart rates over the course of the study were analyzed by repeated analysis of variance (ANOVA). Individual means for body weights, serum levels of glucose, insulin, and lipids and tissue content of glycogen and fat were compared by the t-test. Statistical significance was defined as $p<0.05$.

Results

RT-PCR analysis of cardiac tissue showed the expression of wild type *Cd36* mRNA in both the SHR-TG93 and SHR-TG106 transgenic lines but not in the transgene negative littermates that express only the endogenous mutant *Cd36* (Fig. 1A). Wild type *Cd36* was also expressed in several other tissues including the kidney, liver, white adipose tissue (WAT), and skeletal muscle in both transgenic lines and in the BN rat harboring wild type *Cd36*, but not in the SHR with mutant *Cd36* (Fig. 1B).

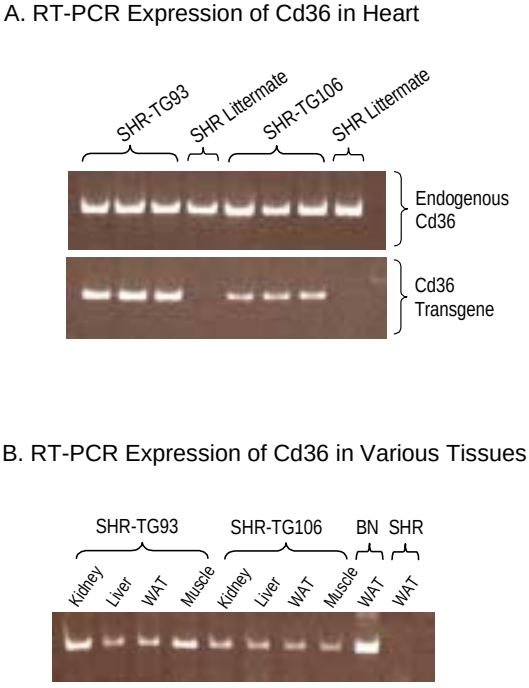

Fig. 1. A: *RT-PCR expression of endogenous mutant and transgenic Cd36 in the heart* **B:** *RT-PCR expression of the wild type Cd36 transgene in the SHR-TG93 and SHR-TG106 transgenic line*

Both the SHR-TG93 and SHR-TG106 lines exhibited significantly decreased serum fatty acid levels when compared to their control littermates (Fig. 2A), but did not differ from their controls in serum glucose, insulin, and triglycerides (data not shown). There were also no significant differences in body weights and in relative weight of the liver, heart, kidneys, and epididymal fat pads between each trangenic line and its control littermate line (data not shown). In addition, the SHR-TG93 line exhibited significantly improved glucose tolerance 120 min after a glucose load (Fig. 2B); the area under the curve (AUC) in the SHR-TG93 transgenic rats versus the SHR controls was significantly smaller (726±8 versus 797±20, $p<0.05$). The SHR-TG106 line showed no significant difference when compared to the SHR-TG negative littermates (Fig. 2B).

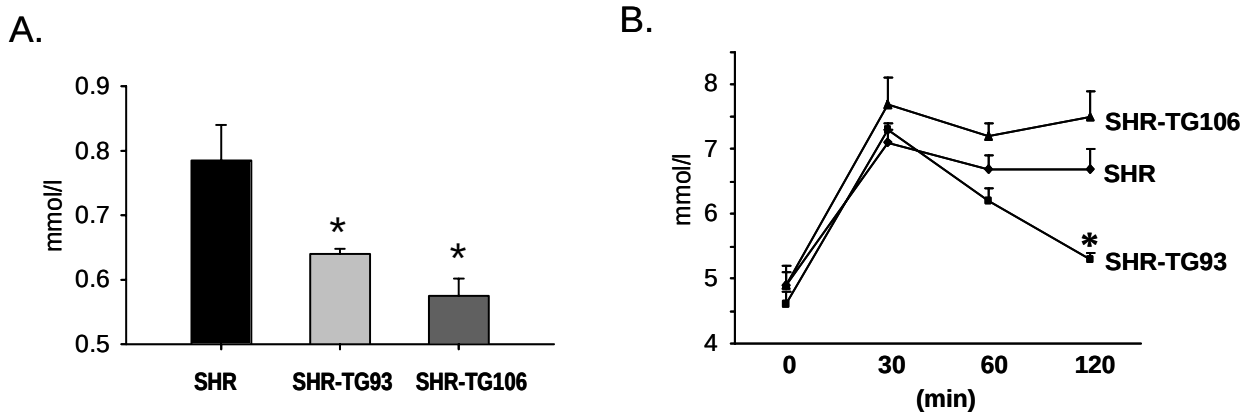

Fig. 2. *Metabolic phenotypes in Cd36 SHR transgenic rats fed a diet with 60% fructose for 15 days. A. Serum non-esterified fatty acids (NEFA). Circulating levels of NEFA in the SHR-TG93 (n = 7) and SHR-TG106 (n = 7) rats were significantly lower when compared to SHR-TG negative control littermates (n = 13). B. Oral glucose tolerance test. * indicates significantly lower (p<0.05) area under the curve in SHR-TG93 rats compared to SHR controls.*

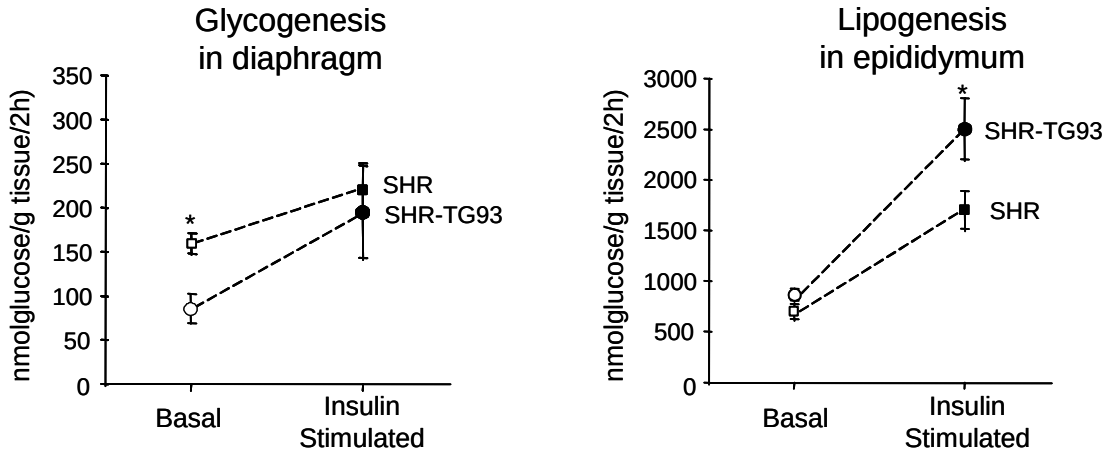

Fig. 3. *Basal and insulin-stimulated glycogenesis in diaphragmatic muscle (left panel) and lipogenesis in epididymal fat (right panel) in the SHR-TG93 transgenic line. Insulin-stimulated incorporation of ^{14}C-U glucose into adipose tissue was significantly greater in the SHR-TG93 transgenic rats than in the SHR-TG negative control littermates. All symbols as in Figure 2.*

Transgenic expression of wild type *Cd36* was also associated with significant amelioration of insulin resistance at the tissue level. Insulin-stimulated glucose incorporation into lipids in epididymal fat pads in the SHR-TG93 line (Fig. 3), or into glycogen in diaphragmatic muscle in the SHR-TG106 line (Fig. 4) were significantly increased when compared to the SHR-TG negative littermates. The variability observed between the SHR-TG93 and SHR-TG106 transgenic lines regarding glucose tolerance and insulin sensitivity on tissue levels could be explained by differences in transgene expression in tissues important for carbohydrate and lipid metabolism, including fat, muscles, or liver. This might be related to the site of insertion and/or to the copy number of the transgene that were integrated into the SHR genome.

Unlike the changes in metabolic phenotypes, expression of the *Cd36* transgene did not cause any differences in mean arterial blood pressures between each transgenic line and its respective control line (Fig. 5).

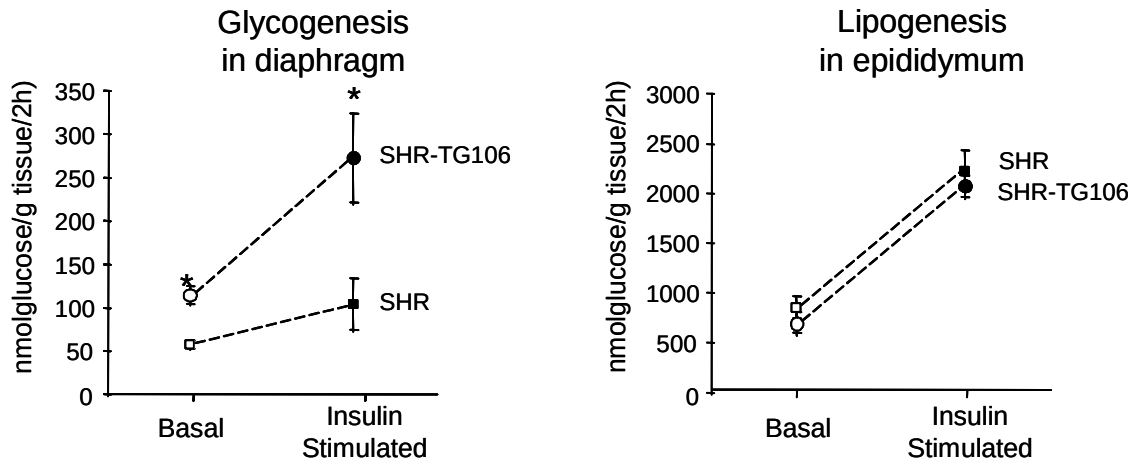

Fig. 4. *Basal and insulin-stimulated glycogenesis in diaphragmatic muscle (left panel) and lipogenesis in epididymal fat (right panel) in the SHR-TG106 transgenic line. Insulin-stimulated incorporation of ^{14}C-U glucose into diaphragmatic muscle glycogen in SHR-TG106 transgenic rats was significantly greater than in SHR-TG negative control littermates. All symbols as in Figure 2.*

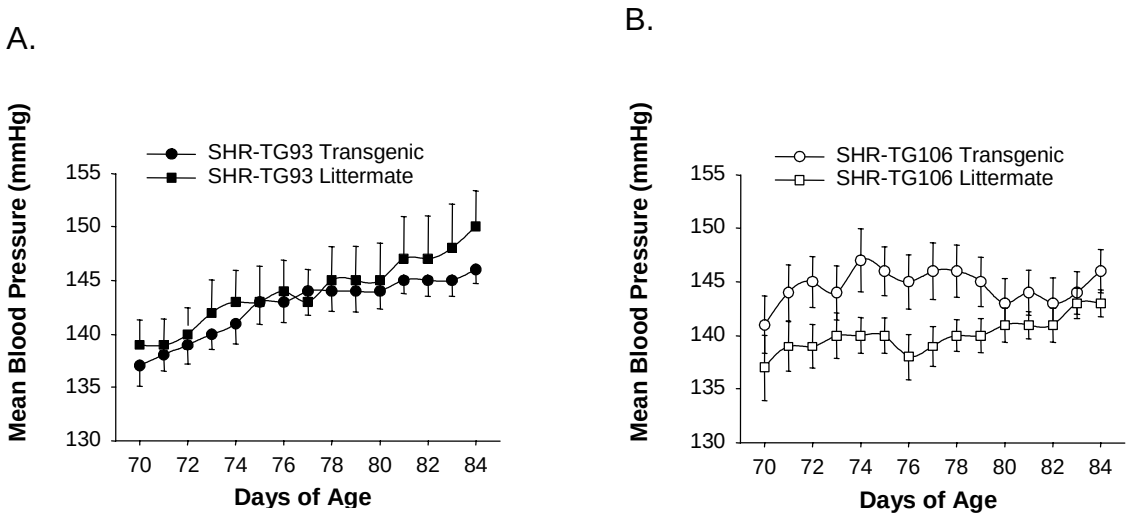

Fig. 5. *24-hour average mean arterial blood pressures determined by radiotelemetry over a period of 2 weeks in the SHR transgenic strains and the SHR-TG negative control littermates. There were no significant differences in mean arterial blood pressure either in the SHR-TG93 (n = 8) (Figure 5A) or in the SHR-TG106 (n = 7) (Figure 5B) transgenic strains when compared to their SHR-TG negative control littermates (n = 8 and 9, respectively)*

Discussion

The current findings provide additional evidence that *Cd36* deficiency may represent a primary defect contributing to altered insulin action and impaired fatty acid metabolism in the SHR/NIH strain. However, transgenic expression of wild type *Cd36* on the SHR background showed little or no effect on blood pressure regulation. Altogether, we have derived four SHR transgenic lines in which wild type *Cd36* was expressed under the control of a universal promoter (lines 10, 19, 93, and 106). Among them, only a single line (SHR-TG19) exhibited a modest decrease in blood pressure. The decreases in blood pressure in the SHR-TG19 line

might be due to the marked overexpression of the *Cd36* transgene observed in the SHR-TG19 kidney (Pravenec *et al.* 2001a) since CD36 could serve as a scavenger receptor which might oppose renal oxidative stress that would otherwise promote increased blood pressure (Makino *et al.* 2002). The possible effect of renal overexpression of *Cd36* on blood pressure regulation can be tested by kidney cross transplantation experiments between histocompatible congenic, transgenic and SHR progenitor strains. In addition, we have derived three SHR transgenic lines with muscle-specific expression of wild type *Cd36* (lines 654, 665, and 669). The blood pressures in these lines are also very similar to those in the SHR progenitor strain (Zídek *et al*. 2002). Recently, Hajri *et al*. (2001) found that supplementation of a diet with short- and medium-chain fatty acids, which are transported into the cytoplasm independently of CD36, normalized metabolic defects but had no effect on increased blood pressure in the SHR. Whereas expression of wild type *Cd36* improved insulin resistance and defective fatty acid metabolism in all of our transgenic lines, the lack of a consistent improvement in blood pressure suggests that *Cd36* deficiency itself is no major determinant of hypertension in the SHR.

The role of CD36 in carbohydrate and fatty acid metabolism as well as cardiovascular function depends on complex genotype-environment interactions. In the SHR strain, significant effects of CD36 deficiency on metabolic phenotypes are noted primarily after feeding a diet high in fructose (Pravenec *et al*. 1999, 2001a). Hajri *et al*. (2002) also demonstrated that the effects of CD36 deficiency on insulin resistance in null C57BL/6 mice are dependent on the carbohydrate composition of the diet. A high-starch, low-fat diet enhanced insulin responsiveness while a high-fructose diet predisposed to insulin resistance. Therefore, it is conceivable that under different dietary conditions such as a high-fructose or a high-fat diet and/or during a longer period of blood pressure measurement, CD36 deficiency might promote increased blood pressure. For example, it is conceivable that CD36 may mediate the inhibitory effects of a high-fat diet on endothelial nitric oxide production with subsequent effects on vascular function and blood pressure (Kincer *et al*. 2002).

In conclusion, transgenic expression of wild type *Cd36* in multiple SHR strains improves insulin sensitivity and dyslipidemia but fails to cause any consistent changes in blood pressure. These findings provide further evidence that the clustering of metabolic risk factors in the SHR model of essential hypertension may be influenced by the pleiotropic effects of defective *Cd36*. However, the association of hypertension with these metabolic risk factors appears to be very complex and is not simply due to the effects of insulin resistance on blood pressure or to SHR defects in the CD36 fatty acid transporter.

Acknowledgements

This work was supported by grants 301/00/1636 and 305/00/1646 from the Grant Agency of the Czech Republic to V.Z. and M.P., grant 4904-3 from the Ministry of Health of the Czech Republic to L.K., NIH grants RO1 HL56028 and PO1 HL35018 (project 2) to T.W.K. and RO1 HL63709 to E.S.L. and T.K., and by a grant RO3 TW01236 from NIH Fogarty Award (FIRCA) to M.P. and T.W.K. M.P. is an International Research Scholar of the Howard Hughes Medical Institute and E.S.L. is a recipient of a Mentored Clinical Scientist Award from the NIH/NHLBI

References

AITMAN TJ, GOTODA T, EVANS AL, IMRIE H, HEATH KE, TREMBLING PM, TRUMAN H, WALLACE CA, RAHMAN A, DORE C, FLINT J, KŘEN V, ZÍDEK V, KURTZ TW, PRAVENEC M, SCOTT J: Quantitative trait loci for cellular defects in glucose and fatty acid metabolism in hypertensive rats. *Nat Genet* **16:** 197-201, 1997.

AITMAN TJ, GLAZIER AM, WALLACE CA, COOPER LD, NORSWORTHY PJ, WAHID FN, AL-MAJALI KM, TREMBLING PM, MANN CJ, SHOULDERS CC, GRAF D, ST LEZIN E, KURTZ TW, KŘEN V, PRAVENEC M, IBRAHIMI A, ABUMRAD NA, STANTON LW, SCOTT J: Identification of Cd36 (Fat) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat Genet* **21:** 76-83, 1999.

BECK-NIELSEN H: General characteristics of the insulin resistance syndrome: prevalence and heritability. European Group for the study of Insulin Resistance (EGIR). *Drugs* **58** (Suppl 1): 7-10, 1999.

ERNSBERGER P, KOLETSKY RJ, FRIEDMAN JE: Molecular pathology in the obese spontaneous hypertensive Koletsky rat: a model of syndrome X. *Ann N Y Acad Sci* **892:** 272-288, 1999.

GROOP L: Genetics of the metabolic syndrome. *Br J Nutr* **83:** S39-S48, 2000.

GROOP L, ORHO-MELANDER M: The dysmetabolic syndrome. *J Intern Med* **250:** 105-120, 2001.

HAJRI T, IBRAHIMI A, COBURN CT, KNAPP FFJR, KURTZ T, PRAVENEC M, ABUMRAD NA: Defective fatty acid uptake in the spontaneously hypertensive rat is a primary determinant of altered glucose metabolism, hyperinsulinemia and myocardial hypertrophy, *J Biol Chem* **276:** 23661-23666, 2001.

HAJRI T, HAN XX, BONEN A, ABUMRAD NA: Defective fatty acid uptake modulates insulin responsiveness and metabolic responses to diet in CD36-null mice. *J Clin Invest* **109:** 1381-1389, 2002.

JENSEN MD: Genetic and environmental contributions. *Nutr Rev* **58**: S22-S24, 2000.

KADOWAKI T: Insights into insulin resistance and type 2 diabetes from knockout mouse models. *J Clin Invest* **106:** 459-465, 2000.

KINCER JF, UITTENBOGAARD A, DRESSMAN J, GUERIN TM, FEBBRAIO M, GUO L, SMART EJ: Hypercholesterolemia promotes a CD36-dependent and endothelial nitric oxide synthase-mediated vascular dysfunction. *J Biol Chem* **277:** 23525-23533, 2002.

KOVACS P, VAN DEN BRANDT J, KLOTING I: Genetic dissection of the syndrome X in the rat. *Biochem Biophys Res Commun* **269:** 660-665, 2000.

MAKINO A, SKELTON MM, ZOU AP, ROMAN RJ, COWLEY AW JR: Increased renal medullary oxidative stress produces hypertension. *Hypertension* **39**: 667-672, 2002.

MAUVAIS-JARVIS F, KAHN CR: Understanding the pathogenesis and treatment of insulin resistance and type 2 diabetes mellitus: what can we learn from transgenic and knockout mice? *Diabetes Metab* **26**: 433-448, 2000.

NEEL JV, WEDER AB, JULIUS S: Type II diabetes, essential hypertension, and obesity as "syndromes of impaired genetic homeostasis": the "thrifty genotype" hypothesis enters the 21st century. *Perspect Biol Med* **42:** 44-74, 1998.

PEDERSEN O: Genetics of insulin resistance. *Exp Clin Endocrinol Diabetes* **107:** 113-118, 1999.

PRAVENEC M, ZÍDEK V, ŠIMÁKOVÁ M, KŘEN V, KŘENOVÁ D, HORKÝ K, JÁCHYMOVÁ M, MÍKOVÁ B, KAZDOVÁ L, AITMAN TJ, CHURCHILL PC, WEBB RC, HINGARH NH, YANG Y, WANG JM, LEZIN ST, LEZIN EM, KURTZ TW: Genetics of Cd36 and the clustering of multiple cardiovascular risk factors in spontaneous hypertension. *J Clin Invest* **103:** 1651-1657, 1999.

PRAVENEC M, ZÍDEK V, LANDA V, KOSTKA V, MUSILOVÁ A, KAZDOVÁ L, FUČÍKOVÁ A, KŘENOVÁ D, BÍLÁ V, KŘEN V: Genetic analysis of cardiovascular risk factor clustering in spontaneous hypertension. *Folia Biol (Praha)* **46:** 233-240, 2000.

PRAVENEC M, LANDA V, ZÍDEK V, MUSILOVÁ A, KŘEN V, KAZDOVÁ L, AITMAN TJ, GLAZIER AM, IBRAHIMI A, ABUMRAD NA, QI N, WANG JM, ST LEZIN EM, KURTZ TW: Transgenic rescue of defective Cd36 ameliorates insulin resistance in spontaneously hypertensive rats. *Nat Genet* **27:** 156-158, 2001a.

PRAVENEC M, LANDA V, ZÍDEK V, KŘEN V: Identification of defective Cd36 as a quantitative trait locus for cardiovascular risk factor clustering in the spontaneously hypertensive rat. *Curr Genomics* **2:** 161-169, 2001b.

REAVEN GM: Role of insulin resistance in human disease (syndrome X): an expanded definition. *Annu Rev Med* **44:** 121-131, 1993

REAVEN GM: Pathophysiology of insulin resistance in human disease. *Physiol Rev* **75:** 473-486, 1995.

ŠEDOVÁ L, KAZDOVÁ L, ŠEDA O, KŘENOVÁ D, KŘEN V: Rat inbred PD/cub strain as a model of dyslipidemia and insulin resistance. *Folia Biol (Praha)* **46:** 99-106, 2000.

ŠTOLBA P, DOBEŠOVÁ Z, HUŠEK P, OPLTOVÁ H, ZICHA J, VRÁNA A, KUNEŠ J: The hypertriglyceridemic rat as a genetic model of hypertension and diabetes. *Life Sci* **51:** 733-740, 1992.

UKKOLA O, BOUCHARD C: Clustering of metabolic abnormalities in obese individuals: the role of genetic factors. *Ann Med* **33:** 79-90, 2001.

VRÁNA A, KAZDOVÁ L: Insulin sensitivity of rat adipose tissue and of diaphragm in vitro: effect of the type of dietary carbohydrate (starch-sucrose). *Life Sci* **9:** 257-265, 1970.

ZÍDEK V, QI N, LANDA V, KŘEN V, KAZDOVÁ L, ST LEZIN E, KURTZ TW, PRAVENEC M: Muscle-specific expression of Cd36 in the SHR is associated with hypertriglyceridemia and glucose intolerance. *ISH Satellite Symposium on the Genetics of Experimental and Human Hypertension*, Brno, 2002 (abstract) p 40.

Reprint requests

Dr. M. Pravenec, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic. E-mail: pravenec@biomed.cas.cz