Table 1. Comparisons of Cd36-null and transgenic mice with a "natural" Cd36-null SHR strain

Strains	HDL cholesterol	Triglycerides	NEFA	Glucose tolerance	Insulin resistance	Blood pressure
Cd36-null mice	↑	↑	↑	↔	↔	?
SHR (vs. SHR-4)	↑	↑	↑	↓	↑	↑
Cd36-transgenic mice	↓	↓	↓	↔	↔	?

↑, ↓, and ↔ denote higher, lower, and unchanged phenotype vs. respective control

overexpression of Cd36 in transgenic mice results in changes in lipid phenotypes that are similar to those observed after transfer of the wild-type Cd36 allele from the BN strain onto the SHR genetic background in the SHR-4 congenic strain.

Relationship of mutations in Cd36 to hypertension

While Cd36 deficiency appears to be a likely determinant of disordered lipid metabolism in the SHR, the role of defective Cd36 in the pathogenesis of spontaneous hypertension and insulin resistance is a far more open question. In demonstrating that the SHR congenic strain has significantly lower BP than the SHR progenitor strain, we have clearly established that the transferred segment of chromosome 4 is involved in the genetic control of hypertension in the SHR-BN model (Pravenec et al., 1999). However, based on genealogic data, the role of Cd36 in the regulation of BP and hypertension is very uncertain. Although the SHR/NIH strain carries the deletion variant in Cd36, we have found that the stroke-prone strain of SHR (SHRSP) did not inherit the SHR deletion variant of Cd36 (the stroke-prone subline branched off from the original SHR line some time after the F10 generation – well after hypertension had been established). Thus, the fact that the SHRSP did not inherit the SHR deletion variant of Cd36 suggests that this gene was not particularly important in the original process of selection for hypertension in the SHR. Other investigators have also found that the original colonies of SHR in Japan do not carry mutant Cd36, further indicating that this mutation arose after the original SHR strain from Kyoto had been transferred to the NIH. However, because the original SHR in Japan and the SHRSP differ from the SHR/NIH strain throughout the entire genome (not just in Cd36), comparisons of these strains are difficult to interpret. Nevertheless, these observations raise serious questions about the potential contribution of Cd36 to the pathogenesis of spontaneous hypertension and insulin resistance.

Development of Cd36-transgenic SHR rats

Linkage studies in SHR have implicated a host of different DNA sequence variants in the genetic control of blood pressure and a variety of other cardiovascular phenotypes. However, direct proof that any of these gene variants represents a QTL regulating blood pressure or any other complex trait is lacking. To directly test the identity of putative QTL in the SHR model, we have: 1) developed transgenic techniques for rescuing mutant alleles directly on the SHR background and 2) used these techniques to determine whether the gene encoding Cd36 truly represents the QTL that can influence the clustering of multiple cardiovascular risk factors in spontaneous hypertension. Towards these ends, we have successfully derived multiple transgenic strains of SHR (SHR-TG strains) that express the wild-type allele for Cd36 on an SHR background harboring the deletion variant of Cd36 (SHR control). In these strains, low-level expression of wild-type Cd36 on the mutant SHR background ameliorated glucose intolerance (e.g., area under the glucose tolerance test curve = 713 ± 21 in the SHR-TG19 strain vs. 954 ± 45 mmol/l/2 h in the SHR control strain, $P < 0.001$), insulin resistance (e.g., insulin-stimulated glucose uptake in muscle = 355 ± 104 in the SHR-TG19 strain vs. 135 ± 13 nmol/g/2 h in the SHR control strain, $P < 0.01$), and dyslipidemia (e.g., fatty acid levels = 276 ± 27 in the SHR strain vs. 389 ± 42 μmol/l in the SHR control strain, $P < 0.05$). Similar results were observed in the SHR-TG10, SHR-TG93, and SHR-TG106 strains. In addition, in the SHR-TG19 strain, increased transgenic expression of wild-type Cd36 in the kidney was associated with significant reductions in BP ($P < 0.005$ by radiotelemetry) (Pravenec et al., 2000). These findings: 1) demonstrate that transgenic SHR can be derived to directly test the identity of putative QTL for complex cardiovascular traits; 2) establish that defective Cd36 is a bona fide QTL that can influence the clustering of multiple cardiovascular risk factors in spontaneous hypertension, and 3) should motivate further studies on the role of Cd36 is the regulation of BP, renal function, and the pathogenesis of hypertension.

References

Aitman, T. J., Gotoda, T., Evans, A. L., Imrie, H., Heath, K. E., Trembling, P. M., Truman, H., Wallace, C. A., Rahman, A., Dore, C., Flint, J., Křen, V., Zídek, V., Kurtz, T. W., Pravenec, M., Scott, J. (1997) Quantitative trait loci for cellular defects in glucose and fatty acid metabolism in hypertensive rats. *Nat. Genet.* **16,** 197-201.

Aitman, T. J., Glazier, A. M., Wallace, C. A., Cooper, L. D., Norsworthy, P. J., Al-Majali, K. M., Trembling, P. M., Mann, C. J., Shoulders, C. C., Graf, D., St.Lezin, E., Kurtz, T. W.,

Křen, V., Pravenec, M., Ibrahimi, A., Abumrad, N. A., Stanton, L. W., Scott, J., (1999) Identification of Cd36 (Fat) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat. Genet.* **21,** 76-83.

Bottger, A., van Lith, H. A., Křen, V., Křenová, D., Bílá, V., Vorlíček, J., Zídek, V., Musilová, A., Zdobinská, M., Wang, J. M., van Zutphen, B. F. M., Kurtz, T. W., Pravenec, M. (1996) Quantitative trait loci influencing cholesterol and phospholipid phenotypes map to chromosomes that contain genes regulating blood pressure in the spontaneously hypertensive rat. *J. Clin. Invest.* **98,** 856-862.

Febraio, M., Abumrad, N. A., Hajjar, D. P., Sharma, K., Cheng, W., Pearce, S. F. A., Silverstein, R. L. (1999) A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J. Biol. Chem.* **274,** 19055-19062.

Hwang, I. S., Ho, H., Hoffman, B. B., Reaven, G. M. (1987) Fructose-induced insulin resistance and hypertension in rats. *Hypertension* **10,** 512-516.

Ibrahimi, A., Bonen, A., Blinn, W. D., Hajri, T., Li, X., Zhong, K., Cameron, R., Abumrad, N. A. (1999) Muscle-specific overexpression of FAT/CD36 enhances fatty acid oxidation by contracting muscle, reduces plasma triglycerides and fatty acids, and increases plasma glucose and insulin. *J. Biol. Chem.* **274,** 26761-26766.

Jacob, H. J., Brown, D. M., Bunker, R. K., Daly, M. J., Dzau, V. J., Goodman, A., Koike, G., Křen, V., Kurtz, T. W., Lernmark, A., Levan, G., Mao, Y., Pettersson, A., Pravenec, M., Simon, J. S., Szpirer, C., Szpirer, J., Trolliet, M., Winer, E. S., Lander, E. S. (1995). A genetic linkage map of the laboratory rat, *Rattus norvegicus. Nat. Genet.* **9,** 63-69.

Pravenec, M., Gauguier, D., Schot t, J., Buard, J., Křen, V., Bílá, V., Szpirer, C., Szpirer, J., Wang, J.-M., Huang, H., St. Lezin, E., Spence, M. A., Flodman, P., Printz, M., Lathrop, G. M., Vergnaud, G., Kurtz, T. W. (1995) Mapping of quantitative trait loci for blood pressure and cardiac mass in the rat by genome scanning of recombinant inbred strains. *J. Clin. Invest.* **96,** 1973-1978.

Pravenec, M., Gauguier, D., Schott, J., Buard, J., Křen, V., Bílá, V., Szpirer, C., Szpirer, J., Wang, J.-M., Huang, H., St. Lezin, E., Spence, M. A., Flodman, P., Printz, M., Lathrop, G. M., Vergnaud, G., Kurtz, T. W. (1996) A genetic linkage map of the rat derived from recombinant inbred strains. *Mamm. Genome* **7,** 117-127.

Pravenec, M., Zídek, V., Šimáková, M, Křen, V., Křenová, D., Horký, K., Jáchymová, M., Míková, B., Kazdová, L., Aitman, T. J., Churchill, P. C., Hingarh, N.H., Yang, Y., Wang, J., St Lezin, E., Kurtz, T. W. (1999) Genetics of Cd36 and the clustering of multiple cardiovascular risk factors in spontaneous hypertension. *J. Clin. Invest.* **103,** 1651-1657.

Pravenec, M., Landa, V., Zídek, V., Křen, V., Kazdová, L., Qi, N., Wang. J., St Lezin, E., Kurtz, T. W. (2000) Transgenic expression of Cd36 attenuates insulin resistance, dyslipidemia, and hypertension in spontaneously hypertensive rats. *Rat Genome* **6,** 60. (Abstract)

Reaven, G., M., Chang, H. (1991) Relationship between blood pressure, plasma insulin and triglyceride concentration, and insulin action in spontaneously hypertensive and Wistar-Kyoto rats. *Am. J. Hypertens.* **4,** 34-38.

Reaven, G. M., Lithell, H., Landsberg, L. (1996) Hypertension and associated metabolic abnormalities – the role of insulin resistance and the sympathoadrenal system. *N. Engl. J. Med.* **334,** 374-381.

Sowers, J. R., Standley, P. R., Ram, J. L., Jacober, S., Sampson, L., Rose, K. (1993) Hyperinsulinemia, insulin resistance, and hyperglycemia: contributing factors in the pathogenesis of hypertension and atherosclerosis. *Am. J. Hypertens.* **6,** S260-S270.

Steen, R. G., Kwitek-Black, A. E., Glenn, C., Gullings-Handley, J., Van Etten, W., Atkinson, O. S., Appel, D., Twigger, S., Muir, M., Mull, T., Granados, M., Kissebah, M., Russo, K., Crane, R., Popp, M., Peden, M., Matise, T., Brown, D. M., Lu, J., Kingsmore, S., Tonellato, P. J., Rozen, S., Slonim, D., Young, P., Knoblauch, M., Provoost, A., Ganten, D., Colman, S. D., Rothberg, J., Lander, E. S., Jacob, H. J. (1999) A high-density integrated genetic linkage and radiation hybrid map of the laboratory rat. *Genome Res.* **9,** AP1-8.