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the appropriate X-only hybrid, mapping the *HNRPG*-homologous structural gene to human and mouse X chromosomes and the retroposons to an autosome (Fig. 1*a*). We confirmed localization to the mouse X chromosome by isolating and FISH mapping a BAC clone (b185) containing the 350-bp fragment amplified by oMJ506/508. This further localized the gene to XA3–XA5 (Fig. 1*b*), consistent with the Xq26 map position for *RBMX* in human[15]. We have named the mouse X gene *Rbmx*. We have shown that *Hnrpg* (ref. 11) is a retroposon derived from *Rbmx*, and it has therefore been renamed *Rbmxrt* (*Rbmx* retrogene). We have localized *Rbmxrt* to mouse chromosome 14 (data not shown).

We isolated and sequenced two mouse *Rbmx* cDNA clones with distinct 3′ ends, but 100% identity to the exonic sequences of the oMJ506/508 genomic product. The first, phnR8c3, was isolated from an adult testis cDNA library using the oMJ506/508 genomic product as probe (Fig. 2*a*, *Rbmx*-2). The second, an adult liver cDNA clone (IMAGE 1891274), was identified via an EST (GenBank AI225991) with 100% identity to the 5′ UTR of *Rbmx*-2 (Fig. 2*a*, *Rbmx*-1). The cDNAs have 100% identity over 1,005 bp, but *Rbmx*-2 lacks the 102 carboxy-terminal amino acids encoded by the 3′ exon of *Rbmx*-1 and has a distinct 3′ UTR. *Rbmx*-1 has the same structure as described *Hnrpg* transcripts. It has 89% nucleotide identity with the published *Hnrpg* sequence[11] and 96% amino acid identity, suggesting that the *Rbmxrt* retrogene has been functionally conserved. We verified the presence of both 3′ ends at the *Rbmx* locus by PCR with DNA from the mouse X-only hybrid and b185 using primer oMJ586 with either oMJ582 (3′ UTR of *Rbmx*-1), which amplifies a 1.4-kb genomic fragment, or oMJ587 (3′ UTR of *Rbmx*-2), which amplifies a 3-kb genomic fragment (data not shown). RT-PCR with the same primer pairs showed that both transcripts are ubiquitously expressed, and this, together with the proposed structure of the mouse *Rbmx* locus, is shown (Fig. 2).

We conclude that *RBMY* is an X-NRY gene. Our results show that the NRY multi-copy testis-specific gene families are of two types. First, those that have been acquired directly by the NRY from an autosomal gene, as exemplified by *DAZ*, and second, those that are X-NRY genes whose transcription has become limited to the testis. The many other X-NRY genes that are still ubiquitously transcribed may therefore function in spermatogenesis only and cannot be excluded as candidate spermatogenesis genes.

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Absence of *Cd36* mutation in the original spontaneously hypertensive rats with insulin resistance

The spontaneously hypertensive rat (SHR) is a model of human insulin-resistance syndromes due to the presence of essential hypertension, hyperinsulinaemia, glucose intolerance, hypertriglyceridaemia and visceral obesity[1–5]. In experimental crosses, quantitative trait loci (QTLs) for SHR defects in glucose and fatty acid metabolism in adipocytes[5], as well as hypertension[6] and hypertriglyceridaemia[7], have been mapped to an overlapping region of rat chromosome 4. Upon analysis of SHR strains derived from a colony at the NIH, a deletional mutation in *Cd36* (formerly *Fat*) was proposed as the underlying cause of these chromosome 4 QTLs and the clustering of insulin resistance phenotypes in SHR (ref. 8). Here we show that the *Cd36* mutation is absent in the original SHR strains, maintained since their development in Japan[9] (SHR/Izm), and question the aetiological relevance of the *Cd36* mutation to insulin resistance in SHR.

We initially searched for genes that were differentially expressed between SHR and its genetic control, the Wistar Kyoto (WKY) rat. Of the 500 independent clones obtained by subtraction analysis of adipocyte cDNA from NIH-derived strains (SHR/NCrj and WKY/NCrj), 2 contained partial cDNA sequences of *Cd36* (ref. 10), the rat homologue of human *CD36*. Northern-blot analysis showed the presence of aberrant *Cd36* mRNA in SHR/NCrj, but not in SHR/Izm (Fig. 1*a*). *Cd36* cDNA from SHR/NCrj contained multiple sequence variants as reported[8], whereas *Cd36* cDNA from SHR/Izm was normal. Genotype analysis demonstrated that the *Cd36* mutation was not present in any of the original SHR/Izm strains, but was found in all NIH-derived strains examined (Fig. 1*b*).

SHR was established as an animal model of genetic hypertension[1] and was sent to the NIH Animal Genetic Resource at the F_{13} generation. Several strains separated from the NIH colony thereafter and were distributed worldwide (Fig. 1*c*). The observed discrepancy with respect to *Cd36* might therefore result from the occurrence of a *de novo* mutation or acquisition of a foreign chromosomal fragment during breeding at the NIH. To explore these possibilities, we compared allele distribution patterns of chromosome 4 markers among SHR strains (Fig. 1*d*). Both the three original

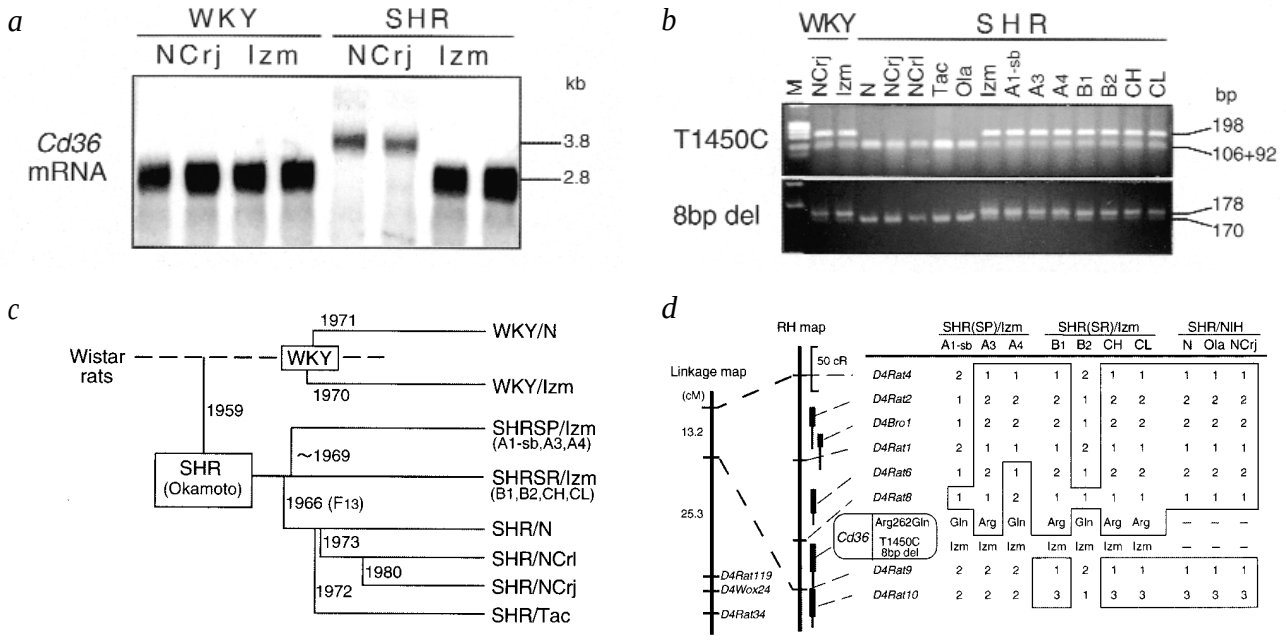
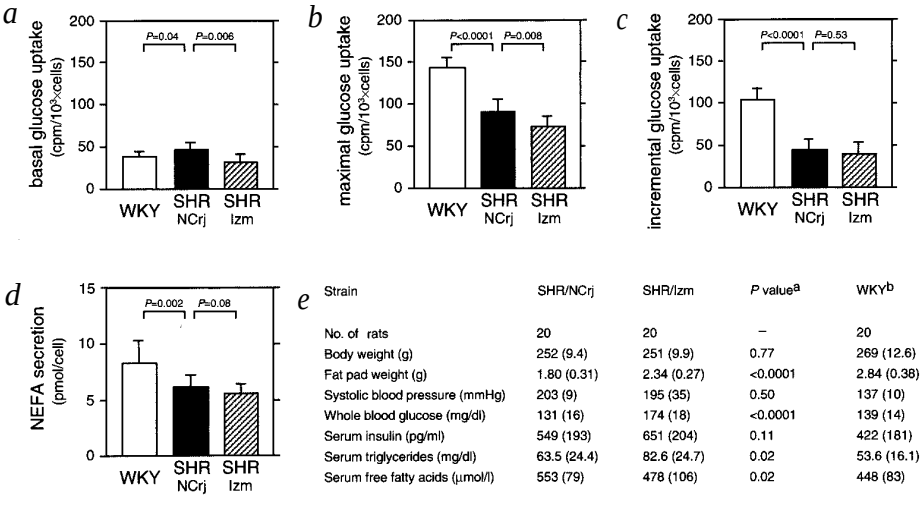


Fig. 1 *Cd36* mRNA, *Cd36* genotypes, genealogical relationships and comparison of chromosomal haplotypes. ***a***, Northern-blot analysis of total RNA (10 μg per lane) isolated from rat adipose tissue with a rat *Cd36* cDNA probe[10] (nt 247–1,570). ***b***, Genotype analysis of PCR products derived from *Cd36* reveals the T1450C variant by digestion with *Bsp*HI (top) and an 8-bp deletion polymorphism (bottom). Rat *Cd36* is at least duplicated, but only one copy is transcribed. In certain substrains of SHR, the functional copy of *Cd36* has a gross deletional rearrangement resulting in the aberrant mRNA splicing[8]. Sequence polymorphisms in the duplicated genes can be exploited to distinguish animals that are homozygous with respect to the *Cd36* mutation; these give rise to a single band on agarose gels. The 8-bp deletion occurs in the putative intron 14 splice-acceptor site of the duplicated copy of the gene that would underlie the aberrant mRNA splicing. Most of the aberrantly spliced mRNAs retained the 5′ sequence of intron 14, possibly due to 'read-through' at the intron 14 splice-donor site (data not shown). M, molecular size marker (ø*x*174/*Hae*III); NCrj, rats from Charles River (Japan); Izm, Funabashi Farm; N, NIH Animal Genetic Resource; NCrl, Charles River (UK); Tac, Taconic; Ola, Prague[6]; A1-sb–CL, substrains from the original Kyoto colony[9]. SHR/NCrl and SHR/Ola were analysed in studies by Aitman *et al*.[5,8]. ***c***, Schematic representation of the origin of SHR and the relationship between substrains. SHR(SP), stroke-prone SHR; SHR(SR), stroke-resistant SHR. ***d***, Comparison of allele distribution pattern among 10 SHR strains. We analysed 32 microsatellite markers surrounding the *Cd36* locus. Only the allele distribution pattern of 8 markers is shown because the other 24 markers provided no additional information. We also analysed 3 markers at the *Cd36* locus, including the novel Arg262Gln and 8-bp deletion polymorphisms. The deleted alleles in SHR/NIH strains are indicated (–). RH, radiation hybrid; SHR/NIH, SHR derived from the NIH colony; cM, centiMorgans; cR, centiRay$_{3,000}$.

(B1, CH, CL) and the NIH-derived SHR strains were shown to share an identical chromosomal haplotype on either side of *Cd36*, supporting the possibility that the *Cd36* mutation was introduced as a *de novo* mutation.

We next studied insulin-stimulated glucose uptake and catecholamine-induced lipolysis in isolated adipocytes, phenotypes used initially to identify the chromosome 4 QTLs (ref. 5). We compared these *in vitro* phenotypes in



e

Strain	SHR/NCrj	SHR/Izm	*P* value[a]	WKY[b]
No. of rats	20	20	–	20
Body weight (g)	252 (9.4)	251 (9.9)	0.77	269 (12.6)
Fat pad weight (g)	1.80 (0.31)	2.34 (0.27)	<0.0001	2.84 (0.38)
Systolic blood pressure (mmHg)	203 (9)	195 (35)	0.50	137 (10)
Whole blood glucose (mg/dl)	131 (16)	174 (18)	<0.0001	139 (14)
Serum insulin (pg/ml)	549 (193)	651 (204)	0.11	422 (181)
Serum triglycerides (mg/dl)	63.5 (24.4)	82.6 (24.7)	0.02	53.6 (16.1)
Serum free fatty acids (μmol/l)	553 (79)	478 (106)	0.02	448 (83)

Fig. 2 Comparison of insulin and catecholamine action in adipocytes and *in vivo* phenotypes. ***a***, Basal glucose uptake before insulin stimulation (50,000 pmol/l). ***b***, Maximal glucose uptake after insulin stimulation. ***c***, Insulin-mediated glucose uptake (maximal minus basal). ***d***, Secretion of non-esterified fatty acids (NEFA) induced by the β-adrenergic agonist isoproterenol (200 nmol/l) in isolated adipocytes from WKY, SHR/NCrj and SHR/Izm (n=10 in each group). Results from WKY/NCrj (n=5) and WKY/Izm (n=5) were combined because they showed no significant differences. Data represent the mean±s.e.m.; cpm, counts per minute. We carried out experiments as described[5], but adipocytes were isolated from epididymal fat pads; these gave results similar to those of retroperitoneal fat pads. Given the postulated function of Cd36 as a fatty acid transporter[10,12], the increased basal glucose uptake observed in adipocytes from SHR/NCrj may be explained by the glucose–fatty acid cycle theory[13]. In fact, impaired use of fatty acids and reciprocal upregulation of glucose uptake have been demonstrated in heart of both SHR/NCrl (ref. 14) and human subjects with CD36 deficiency[15]. ***e***, Comparison of *in vivo* phenotypes. Data are from 12-week-old male rats after an overnight fast. Values are means (s.d.). [a]*P* values are given for the comparison between SHR/NCrj and SHR/Izm. [b]Results are from ten WKY/NCrj and ten WKY/Izm.

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SHR/NCrj and SHR/Izm, which allowed us to examine the biological effects of the Cd36 defect in SHR (Fig. 2*a*–*d*). We observed no significant differences in either phenotype between the two SHR strains (Fig. 2*c*,*d*). We offer two possible explanations for this observation. First, there may be another gene mutation common to both SHR strains, tightly linked to *Cd36*. In this case, the *Cd36* mutation is not the basis of the defective cellular phenotypes observed in SHR. Second, there may be another unlinked genetic difference between the two SHR strains accounting for the cellular phenotypes in SHR/Izm, whereas the Cd36 defect is the cause of these phenotypes in SHR/NCrj. Linkage studies in the SHR/Izm strain will distinguish between these two possibilities.

We also compared the *in vivo* phenotypes associated with insulin-resistance syndrome (Fig. 2*e*). Despite similar body weights and blood pressure levels, SHR/Izm had significantly higher levels of fasting blood glucose, triglycerides and epididymal fat pad weight, which favours SHR/Izm rather than SHR/NCrj as a model of human insulin-resistance syndromes. Supportive *in vivo* evidence obtained by insulin suppression test has been reported[11].

We therefore conclude that the Cd36 defect is unlikely to be a major cause of insulin-resistance phenotypes in SHR. The present study demonstrates the genetic and phenotypic heterogeneity of SHR that should be considered when investigating this important animal model.

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