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Genetics

Rat obesity gene fatty (*fa*) maps to chromosome 5: Evidence for homology with the mouse gene diabetes (*db*)

(Zucker fatty rat/*db* mouse/interferon α/glucose transporter)

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ABSTRACT **The autosomal recessive mutations *fa* (rat) and *db* (mouse) cause obesity syndromes that develop early and ultimately become severe. Although both *fa/fa* rats and *db/db* mice have been studied extensively as models of human obesity and diabetes, the molecular bases of these phenotypes remain unknown. We have mapped *fa* in 50 *fa/fa* (obese) offspring of a (13M × Brown Norway) F_1 *fa/+* intercross relative to two molecular markers, *Ifa* and *Glut-1*, which flank *db* on mouse chromosome 4 and which are located on rat chromosome 5. *Ifa* and *Glut-1* are linked to *fa*, with a gene order, *Ifa–fa–Glut-1*, that is identical to that for the region around *db* in the mouse genome. These results place *fa* on rat chromosome 5 and suggest that *db* and *fa* are mutations in homologous genes.**

Experimental models of obesity are abundant and include seven rodent obesities that are determined by single-gene mutations: yellow (A^y), adipose (*Ad*), diabetes (*db*), fat (*fat*), obese (*ob*), and tubby (*tub*) in the mouse (1) and fatty (*fa*) in the rat (2). The obese phenotypes caused by these mutations differ by age of onset, severity, and degree of insulin resistance and thus present a range of phenotypes similar to that seen in obese humans. Although the molecular bases of these mutations remain unknown, the chromosomal locations of the six mouse obesity genes have been determined (1, 3), and efforts to clone several of these genes by positional genetics have been undertaken (4, 5). As a first step toward cloning the rat gene *fa*, we set out to determine its chromosomal location and here present evidence that it maps to rat chromosome 5 in a region of conserved synteny with the mouse chromosome 4 region containing *db*.

The *fa* mutation arose spontaneously in the Zucker (13M) rat strain (2). Obese (*fa/fa*) rats can be visually distinguished from their lean littermates by 5 weeks of age, although increased adiposity develops earlier (6). Additional aspects of the phenotype include hyperphagia, hyperinsulinemia, hyperlipemia, and multiple endocrine abnormalities (7, 8). A second occurrence of the mutation, originally designated *f* (9) and later renamed *cp* (10), was identified as a *fa* allele (fa^f) by the demonstration that crosses between individuals carrying *fa* and those carrying *f* yield obese progeny (11).

One consideration in mapping *fa* was the prospect that *fa* may be a homolog of one of the mouse obesity genes. Any of the six mouse obesity genes might be considered a candidate for homology with *fa*, but the early onset and severity of obesity mark the *ob* and *db* mutations as most similar to *fa* (1). However, the results of parabiosis experiments have been interpreted as evidence that both *fa/fa* rats and *db/db* mice fail to respond to a circulating factor that suppresses body weight or body fat, whereas *ob/ob* mice appear to lack the activity of this factor (12, 13). Furthermore, mutations at the *db* locus have been recorded on multiple occasions, whereas the *ob* mutation has been noted only once (ref. 14, pp. 85–86). These considerations made *db* the primary candidate for homology with *fa*. Because the mouse and rat share considerable conservation of gene order in the limited number of instances in which regions of these genomes have been compared (J. Nadeau, personal communication), markers closely linked to a given locus in the mouse genome may be expected to be linked to a homologous rat locus. This strategy was used to test loci flanking *db* in the mouse genome for linkage to *fa* in the rat genome.

MATERIALS AND METHODS

Genetic Resource. A cross segregating *fa* was constructed from 13M/Vc *fa/+* male rats (provided by M. R. C. Greenwood, Vassar College, Poughkeepsie, NY) and Brown Norway (BN)/Crl +/+ females (Charles River Breeding Laboratories). BN was chosen as a counterstrain based on historical evidence suggesting that 13M is more distant genetically from BN than it is from other common inbred rat strains (15, 16). Half of the (13M × BN) (13MBN) F_1 offspring of this cross were expected to carry *fa*. The *fa/+* animals were identified by test crosses: random brother × sister matings were made and their F_2 litters were visually screened for the presence of obese pups at 5–6 weeks of age. F_1 crosses that did not produce obese pups were excluded. All breeders and their offspring were maintained on ad libitum water and Purina Rodent Laboratory Chow 5001.

The 13MBN F_2 progeny segregating *fa* were weaned at 21 days of age and separated by sex. Littermates were sacrificed by CO_2 asphyxiation at 50–73 days of age after a 16- to 24-hr fast. Body weight was recorded to the nearest gram. Bilateral inguinal adipose pads were dissected and weighed to the nearest milligram. Liver, spleen, and tail were dissected and frozen at −70°C for subsequent isolation of high molecular weight DNA (17).

Genotype at the *fa* Locus. Accurate identification of genotype at the *fa* locus among F_2 animals was essential for linkage analysis. 13MBN F_2 rats were scored as lean or obese by an index of body fatness: the inguinal adipose tissue weight as a percentage of body weight. This adiposity index divides the 13MBN F_2 population into two nonoverlapping distributions, providing unambiguous identification of *fa/fa* animals (see *Results*).

Genotypes at *Ifa* and *Glut-1*. 13MBN F_2 rats identified as *fa/fa* by phenotype were further characterized for 13M and BN alleles at two additional loci, interferon α (*Ifa*) and facilitative glucose transporter (*Glut-1*)[§], which flank the *db* locus on mouse chromosome 4 (18). The *Ifa* locus was scored for 13M and BN alleles by a restriction fragment length polymorphism (Fig. 1*A*); a 4.0-kilobase band marked the 13M

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[§]We have adopted the mouse genome nomenclature for these loci for the sake of clarity in comparing rat and mouse genomes. However, others have previously applied the human locus designations, *IFNA* and *GLUT1*, to these rat genomic loci (24).

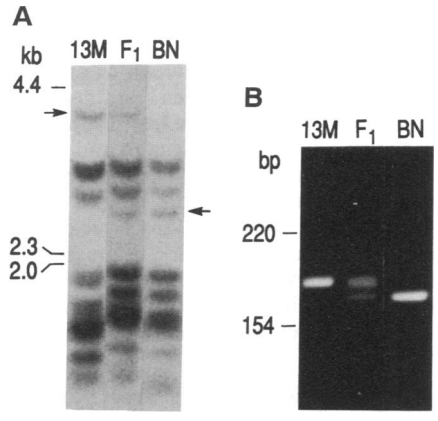

FIG. 1. DNA polymorphisms at two loci linked to *fa*. (*A*) An *Rsa* I restriction fragment length polymorphism for *Ifa*. Parental alleles (13M and BN) are shown in the first and last lanes. Arrows designate the bands chosen to define the parental alleles. The middle lane illustrates the combined restriction fragment length polymorphism pattern found in the F_1 generation. kb, Kilobases. (*B*) An amplified sequence polymorphism for *Glut-1*. Parental alleles are shown in the end lanes; the BN PCR product is a few base pairs (bp) shorter than the 13M PCR product. The heterozygous genotype of the F_1 rats is shown in the middle lane.

allele and a 2.7-kilobase band indicated the BN allele. DNA (10 μg) from each rat was digested with 30 units of restriction endonuclease *Rsa* I (Boehringer Mannheim) and electrophoresed through 1.5% LE agarose (Sigma) at 0.7 V/cm for 20–22 hr in TAE (40 mM Tris acetate/1 mM EDTA). For Southern blot analysis, DNA was transferred from the gel to nylon membranes (GeneScreen*Plus*, NEN) with 0.4 M NaOH as the transfer buffer. Blots were neutralized in 0.2 M Tris·HCl, pH 7.5/2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), and DNA was fixed to the membrane by drying. Blots were prehybridized for 2 hr at 65°C with 10% (wt/vol) dextran sulfate/1 M NaCl/1% SDS/denatured salmon sperm DNA (≈100 μg/ml). The *Ifa* gene probe, an ≈800-base-pair *Eco*RI–*Hin*dIII fragment of pKK12 (19), generously provided by P. Pitha-Rowe, was labeled with [^{32}P]dCTP by the random-primer method (20) and added to the hybridization mixture at 10^6 dpm/ml. After 16–20 hr at 65°C, blots were washed briefly with 2% SDS/2× SSC and then washed twice at 65°C for 20–30 min in 2% SDS/2× SSC. Blots were exposed to Kodak X-Omat AR film for 24–72 hr at 70°C before developing.

The 13MBN F_2 *fa/fa* rats were scored for 13M and BN alleles at *Glut-1* by an amplified sequence polymorphism (Fig. 1*B*); an ≈185-base-pair band marked the 13M allele and an ≈195-base-pair band indicated the BN allele. Oligonucleotide primers (GAATGAAGCTAAGAATTGACCTTAGGT and GTCCATGCCTGTCCTTTAGTGCTCTTG) were constructed to flank a CA repeat within intron H of *Glut-1* (21). Intervening sequences were amplified from 250 ng of DNA in a solution containing each primer at 0.1 μg, 50 mM KCl, 10 mM Tris Cl (pH 8.3 at 27°C), 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 200 μM dTTP, and 0.5 unit of *Taq* polymerase (GeneAmp, Perkin–Elmer/Cetus). Thirty cycles were carried out at 94°C to denature, 50°C to anneal, and 72°C to extend; each cycle segment lasted 1 min initially and the third segment was extended by 3 sec per cycle. Amplification products were electrophoresed in 6% NuSieve 3:1 (FMC) in TAE at 1 V/cm, stained with ethidium bromide, and photographed under UV illumination.

RESULTS

Identification of *fa/fa* Rats. The adiposity index divides the 13MBN F_2 population into two nonoverlapping distributions, providing unambiguous identification of *fa/fa* individuals (Fig. 2). The adiposity index values (mean ± SEM) were 0.71 (±0.02) for the lean (+/+ or *fa*/+) rats in the lower cluster and 4.44 (±0.11) for the obese (*fa/fa*) rats in the upper cluster. The difference between these means is highly significant ($t = 32.1$; $P < 10^{-6}$). By this criterion, 50 obese and 114 lean rats were identified. Eight rats were not phenotyped because of early death or missing data. The frequency of obese individuals (50/164 = 0.328) was not significantly different from the value of 0.25 predicted for a fully penetrant autosomal recessive gene ($\chi^2 = 2.63$; $P > 0.10$). Because *fa*/+ and +/+ rats are both lean and are not readily distinguishable, only *fa/fa* rats were used for linkage analysis.

Genotypes at *Ifa* and *Glut-1*. The 50 13MBN F_2 *fa/fa* rats were scored for 13M and BN alleles at *Ifa* and *Glut-1* by the molecular polymorphisms illustrated in Fig. 1. In the 50 DNA samples, representing 100 meioses, the restriction fragment length polymorphism for *Ifa* and the amplified sequence

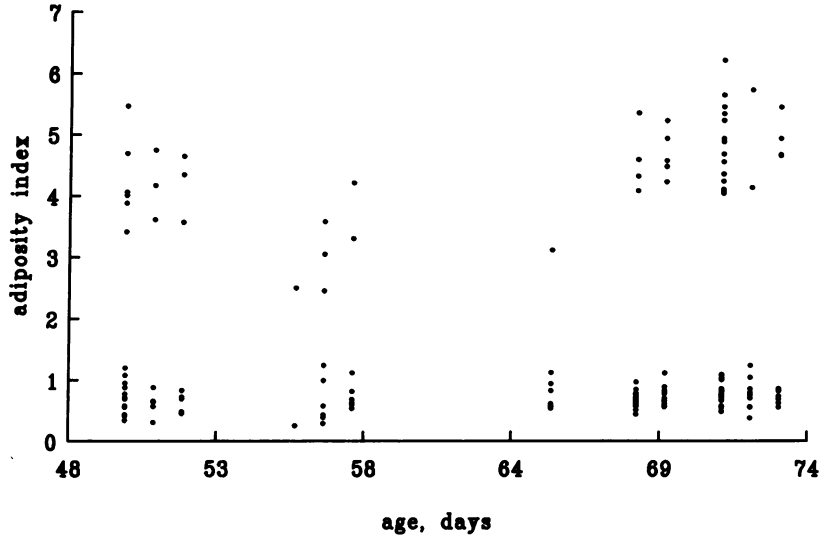

FIG. 2. Scatter plot of the adiposity indices of 13MBN F_2 rats segregating *fa*. The adiposity index was calculated as (inguinal adipose tissue weight/body weight) × 100. A total of 164 values are plotted; 50 in the upper cluster, designating obese (*fa/fa*) rats, and 114 in the lower cluster, designating lean (*fa*/+ or +/+) rats. Note the complete separation of the two clusters; the lowest adiposity index among the obese rats is >8 standard deviations from the mean of the lean rats.

polymorphism for *Glut-1* identified six and seven BN alleles, respectively. No individual animal inherited more than a single BN allele at these loci.

Linkage Analysis. Linkage, gene order, and map distances between *Ifa*, *fa*, and *Glut-1* were derived by pairwise comparisons of recombination frequencies between the loci. Because the 50 obese rats are homozygous for 13M alleles at the *fa* locus, recombinations are marked by BN alleles at *Ifa* or *Glut-1*. The recombination fractions between each pair of loci are significantly less than 50/100 (Table 1), indicating that *Ifa*, *fa*, and *Glut-1* constitute a linkage group. Furthermore, the recombination fractions for *Ifa–fa* and *fa–Glut-1* sum to give the recombination fraction for *Ifa–Glut-1*, resulting in the gene order *Ifa–fa–Glut-1*. The two alternative gene orders, *Ifa–Glut-1–fa* and *fa–Ifa–Glut-1*, require the assumption of seven and six undetected double recombinations and are, therefore, highly unlikely (22).

DISCUSSION

Recent evidence indicates that *Ifa*, *Glut-1*, and 10 other loci that map to mouse chromosome 4 are located on rat chromosome 5 (24). Our linkage data indicate that *fa* is flanked 6.4 and 7.5 centimorgans (cM) to either side by *Glut-1* and *Ifa* (Table 1); placing *fa* on rat chromosome 5 also. In comparison, the *db* locus is flanked proximally ≈3 cM by *Ifa* and 8–10 cM distally by *Glut-1* (18) in the mouse genome. The map distances for rat and mouse genomes should not be considered directly comparable; in addition to species differences, the map distances are likely to vary due to the structure of the genetic crosses used. These data, considered with the similar phenotypes of *fa/fa* rats and *db/db* mice, suggest that *fa* and *db* are mutations in homologous genes and that the *Ifa–fa/db–Glut-1* linkage group is conserved between rat and mouse.

The suggestion that *fa* and *db* are homologs is supported by the similarity of the obesity syndromes seen in *db/db* mice and *fa/fa* rats. Both mutations are recessive and result in early-onset progressive obesity (1, 2). Both phenotypes are accompanied by hyperphagia and increased energy efficiency (25, 26); even when hyperphagia is controlled by pair-feeding obese animals to their lean counterparts, *fa/fa* rats and *db/db* mice store a greater amount of fat (25, 27). Insulin resistance accompanies the development of obesity in these animals (27, 28) and leads to type II diabetes on some genetic backgrounds (29, 30).

The likelihood that *fa* and *db* are homologs provides a unique opportunity to combine the complementary advantages of mouse genetics and rat physiology in exploring the action of these genes. For example, the availability of linked molecular genetic markers for now makes it possible to determine the genotype of preobese rats in the earliest days of life or prenatally, before the development of biochemically confounding secondary features of the phenotype. Also, the genetic cross described here constitutes an ideal resource for testing candidate genes for *fa*.

Confirmation of homology between *fa* and *db* awaits the molecular cloning of these genes. Mutations at *db* have occurred at least five times in mice (ref. 14, pp. 265–266). The availability of two additional alleles (*fa* and *fa*^{f}) in a separate genus may aid this cloning effort. The multiple occurrence of mutations at this locus in separate genera underscores the possibility that similar mutations may have occurred in other species, including man. If a homolog of the *fa/db* gene occurs in man, it is most likely in chromosome 1p31–34, a region of the human genome that has synteny homology with the region of the mouse genome containing the *db* locus (31).

Table 1. Linkage data for three loci mapped in 50 13MBN F_2 rats

Paired loci	Recombination fraction	Map distance, cM
Ifa–fa	6/100	6.4 ± 2.4
fa–Glut-1	7/100	7.5 ± 2.7
Ifa–Glut-1	13/100	15.1 ± 3.6

Recombination fraction is the number of meiotic recombinations detected divided by the number of meioses scored. The observed recombination fractions all differ from 50/100 at $P < 0.00001$ by the χ^2 test. Map distances, expressed as mean ± SEM, were adjusted by the Haldane function (23) to correct for undetected multiple crossovers. cM, centimorgans.

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