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Fine Mapping of the Friend Retrovirus Resistance Gene, *Rfv3*, on Mouse Chromosome 15

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***Rfv3* is a host resistance gene that operates through an unknown mechanism to control the development of the virus-neutralizing antibody response required for recovery from infection with Friend retrovirus. The *Rfv3* gene was previously mapped to an approximately 20-centimorgan (cM) region of chromosome 15. More refined mapping was not possible, due to a lack of microsatellite markers and leakiness in the *Rfv3* phenotype, which prevented definitive phenotyping of individual recombinant mice. In the present study, we overcame these difficulties by taking advantage of seven new microsatellite markers in the *Rfv3* region and by using progeny tests to accurately determine the *Rfv3* phenotype of recombinant mice. Detailed linkage analysis of relevant crossovers narrowed the location of *Rfv3* to a 0.83-cM region. Mapping of closely linked genes in an interspecific backcross panel allowed us to exclude two previous candidate genes, *Ly6* and *Wnt7b*. These studies also showed for the first time that the *Hsf1* gene maps to the Rfv3-linked cluster of genes including *Il2rb*, *Il3rb*, and *Pdgfb*. This localization of *Rfv3* to a region of less than 1 cM now makes it feasible to attempt the cloning of *Rfv3* by physical methods.**

Infection with Friend virus complex (FV) induces rapid polyclonal erythroid cell proliferation and splenomegaly in genetically susceptible adult mice (5, 10). Mice of strains which mount rapid humoral and cell-mediated FV-specific immune responses spontaneously recover from FV-induced splenomegaly without progressing to erythroleukemia. Such recovery from FV disease is dependent on a number of host genes, including several genes of the major histocompatibility complex (MHC), which influence critical $CD4^+$- and $CD8^+$-T-cell responses (5, 13). However, unlike some other viral systems in which either a cellular or humoral immune response alone is sufficient to resolve infection (1, 19, 20), spontaneous recovery from FV requires both FV-specific T-cell responses and virus-neutralizing antibody responses (6, 12, 21).

The non-MHC gene *Rfv3* influences the ability of mice to mount FV-neutralizing antibody responses following infection (6). C57BL/10 and C57BL/6 mice have the genotype $Rfv3^r/Rfv3^r$, and BALB/c, A.BY, and A/WySn mice have the genotype $Rfv3^s/Rfv3^s$ (6, 7, 9). At about 2 weeks postinfection, mice carrying at least one dominant *Rfv3* resistance allele ($Rfv3^r$), such as (C57BL/10 × A.BY)F_1 mice, begin to make FV-neutralizing antibodies, and they usually clear FV plasma viremia by 30 days postinfection (DPI). In contrast, mice with two sensitive alleles ($Rfv3^s/Rfv3^s$) fail to make FV-neutralizing antibodies, remain viremic, and eventually succumb to FV-induced erythroleukemic splenomegaly (6, 9). Interestingly, $Rfv3^s/Rfv3^s$ mice have normal antibody responses to other antigens, suggesting that these mice are not generally immunosuppressed (18). The mechanism whereby *Rfv3* controls the FV-specific humoral response remains unknown. The gene has been mapped to a 20-centimorgan (cM) region of mouse chromosome 15, ruling out linkage to genes such as MHC genes, immunoglobulin genes, or T-cell receptor genes (14). In this study, we used progeny testing and microsatellite linkage analysis with seven new markers to define the location of the *Rfv3* gene to a region of less than 1 cM. These experiments determined that the previous candidate genes, *Ly6* and *Wnt7*, mapped to regions adjacent to, rather than within, the *Rfv3* gene region.

To map the *Rfv3* gene, heterozygous (B10.A × A/Wy)F_1 mice ($Rfv3^r/Rfv3^s$) were intercrossed to produce F_2 offspring. Tail tip DNA samples from 181 F_2 mice were analyzed by using PCR amplification of simple sequence length polymorphisms (microsatellites) (15). Initially, two markers, *D15Mit28* and *D15Mit108*, which flank the 20-cM region containing the *Rfv3* gene (14), were used to identify 45 recombinant F_2 mice. DNA from these recombinants with microsatellite markers lying between the flanking markers was further tested, and nine recombination locations (groups I through IX) were identified (Fig. 1). Because the *Rfv3* phenotype shows some leakiness even in genetically identical mice (Fig. 1) (14), we could not rely on the accuracy of phenotyping of individual recombinant F_2 mice. Rather, the recombinant F_2 mice were backcrossed to A/Wy parental mice, and the resulting progeny were genotyped and tested. A total of 23 of the 45 recombinant F_2 mice were backcrossed, and all progeny were genotyped prior to phenotypic analysis. For determination of the *Rfv3*

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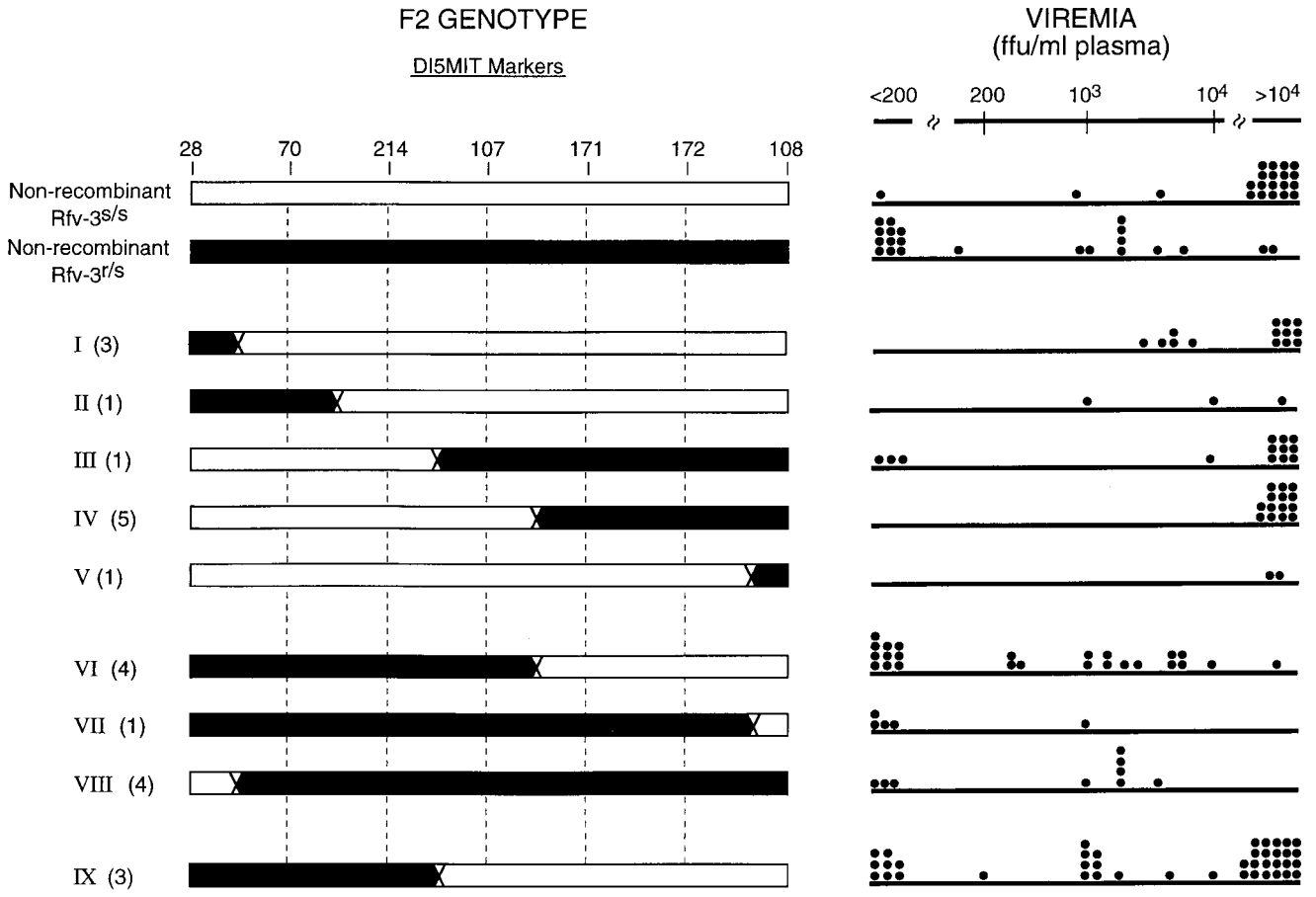

FIG. 1. Genotypes of recombinant (B10.A × A/Wy)F_2 mice and corresponding *Rfv3* phenotypes for (B10.A × A/Wy)F_2 × A/Wy backcross progeny. (Left) F_2 mice were typed for *D15Mit* markers by PCR. Thirty cycles of PCR were performed with 100 ng of tail DNA template, 1× PCR buffer (Promega), 0.2 μM deoxynucleoside triphosphate, 1 mM $MgCl_2$, 1 μM flanking primers, and 0.05 U of *Taq* polymerase (Promega). Arabic numbers at the top refer to the markers used in this study. The nine crossover locations detected on chromosome 15 are shown. The markers are evenly spaced for convenience, and crossover is arbitrarily shown halfway between each marker. Black regions denote DNA originating from the B10.A (*Rfv3*^{r}/*Rfv3*^{r}) parent; white regions denote DNA originating from the A/Wy (*Rfv3*^{s}/*Rfv3*^{s}) parent. Numbers in parentheses indicate the numbers of recombinant F_2 mice showing crossover in the same region which were progeny tested. (Right) Plasma viremia data were analyzed at 30 DPI for individual recombinant (B10.A × A/Wy)F_2 × A/Wy backcross progeny from each crossover group. Viremia data for nonrecombinant (*Rfv3*^{r}/*Rfv3*^{s} or *Rfv3*^{s}/*Rfv3*^{s}) littermates from each backcross are grouped at the top. Viremia levels between 200 and 10^4 FFU/ml are plotted on a $\log_{10}$ scale; values of <200 and values of >10^4 are grouped. Each dot represents the FV viremia titer for one mouse as detected by focal immunoassay on *Mus dunni* cells (17). The detection limit of the assay was 200 FFU/ml.

phenotype, recombinant progeny and a number of nonrecombinant littermate control mice were infected with FV and tested for plasma viremia at 30 DPI. Viremia has been shown to inversely correlate with FV-neutralizing antibody production (9, 22) and is a convenient assay for determining the *Rfv3* phenotype. Mice exhibiting less than 200 focus-forming units (FFU)/ml of plasma were scored as nonviremic, whereas mice with more than 10^4 FFU/ml of plasma were considered highly viremic. Leakiness in the *Rfv3* phenotype was manifested by intermediate viremia levels, which were observed at a low incidence in both *Rfv3*^{r}/*Rfv3*^{s} and *Rfv3*^{s}/*Rfv3*^{s} controls (Fig. 1).

To map *Rfv3*, we looked for a correlation between the genotypes and phenotypes in the recombinant backcross progeny. High levels of viremia were seen in 35 of 46 (76%) recombinant progeny from groups I, II, III, IV, and V. When the genotypes of these mice were compared, it was observed that the mice had the *Rfv3*^{s}/*Rfv3*^{s} genotype at marker *D15Mit214* (Fig. 1). In contrast, the low-viremia groups VI, VII, and VIII had the *Rfv3*^{r}/*Rfv3*^{s} genotype at this marker (Fig. 1). These results indicated that the *Rfv3* gene was located near *D15Mit214* in a region between markers *D15Mit70* and *D15Mit107*.

Group IX, which consisted of progeny from three F_2 recombinants, R45, R172, and R80, exhibited both high and low levels of viremia (Fig. 1 and 2). This variability prompted further typing of the mice of this group in order to detect possible differences in recombination positions. Typing with three markers located between *D15Mit214* and *D15Mit107* revealed unique crossover positions in these three recombinants (Fig. 2). Interestingly, 17 of 18 progeny of R45 were either nonviremic or showed only intermediate viremia (<10^4 FFU/ml of plasma), whereas 20 of 21 progeny of recombinants R172 and R80 were highly viremic (Fig. 2). These data were consistent with a location for *Rfv3* that was distal to *D15Mit239* and proximal to *D15Mit107* (Fig. 2). Progeny testing of another recombinant mouse, R8 (group III), with a recombination in the same region supported this position for *Rfv3* (Fig. 2).

The genotyping of recombinant F_2 mice has allowed us to determine the genetic distances between the *D15Mit* markers used in this study. Our previous results indicated that *Rfv3*

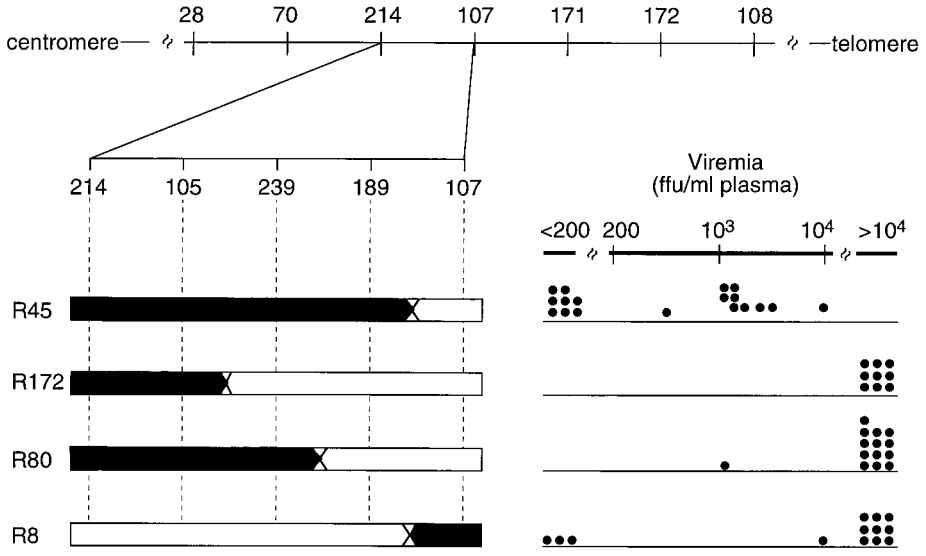

FIG. 2. Fine mapping of the recombination between *D15Mit214* and *D15Mit107* in recombinant F_2 mice (R45, R172, R80, and R8) and *Rfv3* phenotype (viremia) data for recombinant backcross progeny. Genotyping data for the intervening markers *D15Mit105*, *D15Mit239*, and *D15Mit189* are shown. The parental contribution of the recombinant chromosomes is shown as in Fig. 1. Viremia data are the same as those shown in Fig. 1 for the backcross progeny of group IX (R45, R172, and R80) and group III (R8).

was located in a 5- to 20-cM region between *D15Mit108* and *D15Mit93*. Here, we genotyped F_2 mice with the microsatellite markers *D15Mit108* and *D15Mit28*, which is very closely linked to *D15Mit93*. We now estimate the distance between these markers to be 12.2 cM (Fig. 3A). Furthermore, our mapping of *Rfv3* between *D15Mit239* and *D15Mit107* localizes the *Rfv3* position to a 0.83-cM region of chromosome 15 (Fig. 3A).

To identify other genes in the interval of the *Rfv3* locus, we mapped two closely linked markers, *D15Mit214* and *D15Mit239*, in an interspecific backcross panel derived from the matings of (C57BL/6J × *Mus spretus*)F_1 × C57BL/6J mice (8). This mapping panel has been typed for over 2,700 loci, most of which are genes that are well distributed among all the autosomes as well as the X chromosome. By this analysis, *Rfv3* was separable from *Ly6* and *Wnt7b* (Fig. 3B), two genes which cosegregated with *Rfv3* in previous experiments (14). However, *Rfv3* colocalized with a cluster of genes including the immune-system-related genes *Il2rb* (interleukin 2 [IL-2] receptor beta), *Il3rb1* (IL-3 receptor beta 1), and *Pdgfb* (platelet-derived growth factor beta) in the central region of mouse chromosome 15 (3, 4, 11). This cluster of loci is 0.9 cM distal to *Ly6* and 3.1 cM proximal to *Wnt7b* (Fig. 3B).

One previously unmapped gene, *Hsf1* (heat shock factor 1), was also found to map to the same gene region as *Rfv3*. The position of *Hsf1* was determined by Southern blot analysis with a cDNA probe (16). Major fragments of 11.5 and 2.8 kb were detected in *Taq*I-digested C57BL/6J DNA, and major fragments of 5.2 and 3.1 kb were detected in *Taq*I-digested *M. spretus* DNA. The presence or absence of the *M. spretus* fragments which cosegregated was monitored in [(C57BL/6 × *M. spretus*) × C57BL/6]B_1 backcross mice. Recombination distances were calculated with Map Manager, version 2.6.5, and the gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

Ideally, it would be desirable to type the critical recombinant mice for crossovers between *Rfv3* and the candidate genes *Il2rb*, *Il3rb1*, *Pdgfb*, and *Hsf1*. However, to our knowledge, no polymorphisms that distinguish the alleles of these genes in the C57BL/10 and A/Wy strains of mice have been reported. Furthermore, no crossovers between any of these genes have been detected in several crosses including *Mus musculus* and *M. spretus*, where distinguishing between alleles is more feasible. Because of the obvious importance of IL-2 receptor-mediated signal transduction in many immune responses, we made a preliminary attempt to detect allele-specific polymorphisms in *Il2rb* mRNAs but found no allelic differences between C57BL/10 and A/Wy mice. Also, we found no significant differences in the levels of expression of *Il2rb* when spleen RNA was examined by RNase protection assays at multiple time points during the first 3 weeks following infection with FV (data not shown).

The present study has narrowed the location of *Rfv3* by a factor of over 20 and has both excluded two previous candidate genes and included *Hsf1* as a new candidate. The central region of mouse chromosome 15 has homology with human chromosomes 8q and 22q (Fig. 3B), and further mapping of these regions of the human chromosomes may uncover additional candidate genes. While the analysis of candidates is appealing, the *Rfv3* gene region could contain numerous unidentified genes, any of which could be *Rfv3*. The real advantage of the current fine mapping is that identification of *Rfv3* by physical means is now feasible. Bacterial artificial chromosomes containing overlapping sections of the *Rfv3* region can be produced and used to breed transgenic mice. The future identification of the *Rfv3* gene will aid our understanding of the control of the FV host immune response and may ultimately contribute more generally to understanding human retroviral immunity.

The first two authors contributed equally to this study.

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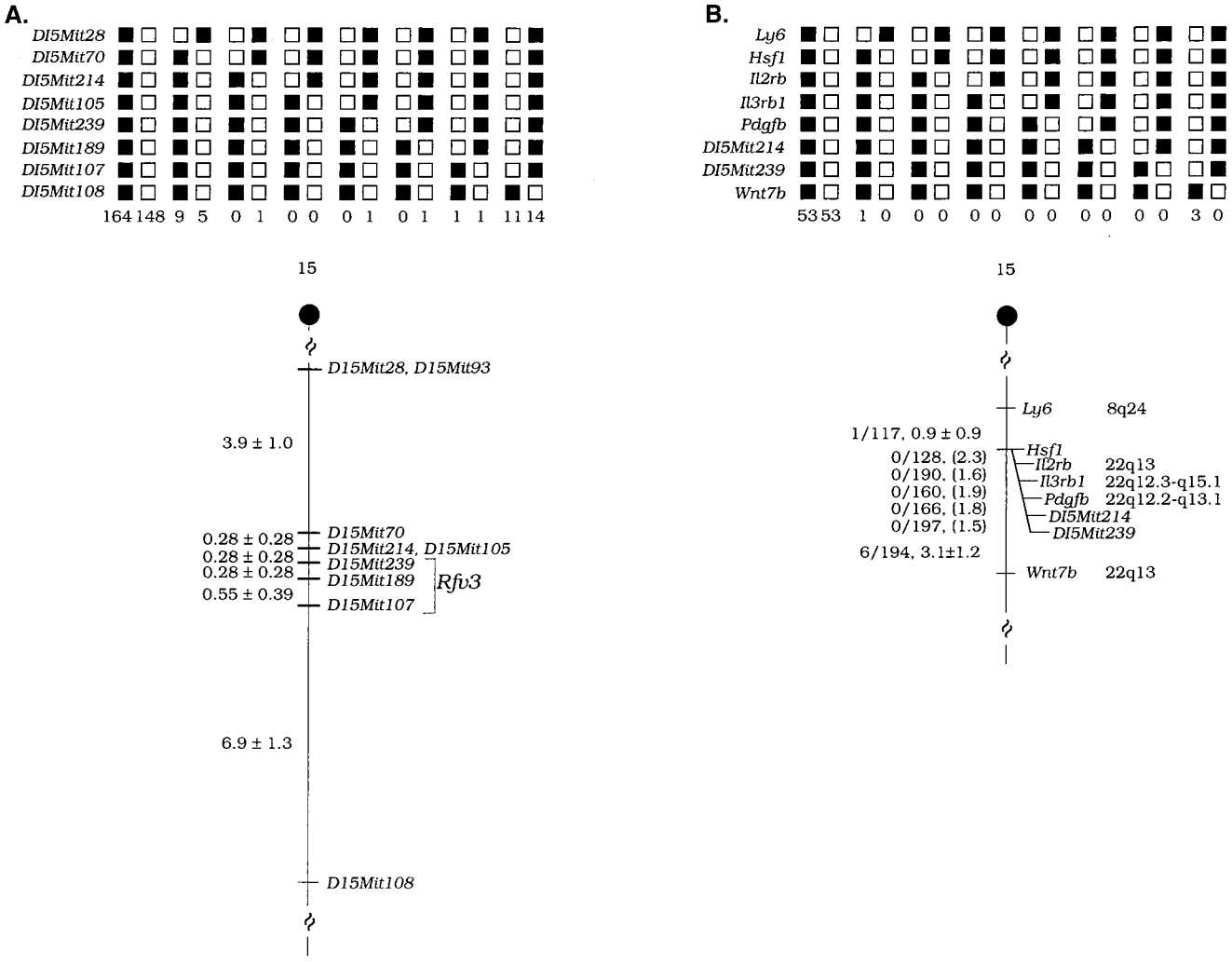

FIG. 3. (A) Genetic distances and predicted position of *Rfv3* on mouse chromosome 15 based on (B10.A × A/Wy)F_2 recombinant mice. The chromosomes inherited from each F_1 parent are shown as columns at the top. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. Shaded boxes represent the presence of a B10.A allele, and white boxes represent the presence of an A/Wy allele. In the partial chromosome 15 linkage map at the bottom, the genetic distances (means ± standard errors) between the *D15Mit* markers shown were calculated as the percentages of (B10.A × A/Wy)F_2 recombinant animals of the total of 181 F_2 animals which were typed. Since each F_1 parent could contribute a recombinant chromosome, the distances were based on 362 total chromosomes 15. Three mice (six chromosomes) were determined to be recombinant in chromosome 15 but were not typeable at all the loci shown, and so they were omitted from the figure. The *Rfv3* gene is predicted to be between *D15Mit239* and *D15Mit107*, based on viremia data for animals in groups IX and III (Fig. 2). (B) *D15Mit214* and *D15Mit239*, markers closely linked to *Rfv3*, map in the central region of mouse chromosome 15. The markers were placed on mouse chromosome 15 by analysis of the progeny of the interspecific backcross (C57BL/6J × *M. spretus*)F_1 × C57BL/6J (8). Only animals that were recombinant in the *Ly6*-to-*Wnt7b* interval of mouse chromosome 15 were typed by PCR under the conditions described in the legend to Fig. 1. The segregation patterns of the markers and flanking genes in 110 backcross animals that were typed for all loci are shown at the top. Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6J × *M. spretus*)F_1 parent. The shaded boxes represent the presence of a C57BL/6J allele, and the white boxes represent the presence of an *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. A partial chromosome 15 linkage map showing the location of *D15Mit214* and *D15Mit239* in relation to linked genes is shown at the bottom. For individual pairs of loci, more than 110 animals were typed. The number of recombinant animals over the total number of animals typed is shown as a ratio; the recombination frequency, expressed as the mean genetic distance in centimorgans (± standard error), is shown for each pair of loci to the left of the chromosome. Standard errors were determined as described previously (2). When no recombination was detected between the loci, the upper 95% confidence limit of the recombination distance is given in parentheses. The positions of the loci in human chromosomes, where known, are shown to the right. References for the human map positions of loci cited in this study can be obtained from the Genome Data Base, a computerized database of human linkage information maintained by the William H. Welch Medical Library of The Johns Hopkins University (Baltimore, Md.).

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