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Genetic Mapping of the *Mx* Influenza Virus Resistance Gene within the Region of Mouse Chromosome 16 That Is Homologous to Human Chromosome 21

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A total of 318 progeny from four backcrosses involving different laboratory strains and subspecies of *Mus musculus* were analyzed to map the *Mx* gene to the region of mouse chromosome 16 (MMU 16) which is homologous to human chromosome 21 (HSA 21). This result suggests that *Mx* will be found in the region of HSA 21 which has been implicated in Down syndrome when inherited in three copies.

The *Mx* gene is responsible for the resistance of some strains of mice to orthomyxovirus infection (9, 11). The expression of *Mx* is tightly regulated by alpha and beta interferons (19), the receptor for which is encoded by a gene located on mouse chromosome 16 (MMU 16) and on human chromosome 21 (HSA 21) (mouse, *Ifrc*; human, *IFREC*) (2, 10). Three transcriptionally active alleles of *Mx* have been reported. The Mx^+ allele encodes a nuclear protein of approximately 72 kilodaltons which is responsible for the viral resistance phenotype (8). Chromosomal DNA analysis has shown that the Mx^+ allele consists of 14 exons distributed over at least 55 kilobases of DNA (9a). Two *Mx* mutant alleles produce nonfunctional transcripts, and mice homozygous for these alleles are susceptible to influenza virus infection. The Mx^- phenotype of strain BALB/cJ is due to a deletion of three exons from the genome, while that of CBA/J probably arises from a nonsense mutation in the *Mx* coding region (19a). The majority of laborator strains carry the BALB/cJ-type *Mx* mutant allele, which can be distinguished from Mx^+ and the CBA/J *Mx* mutation on the basis of restriction fragment length polymorphisms (RFLPs) (21). The genetics, physiology, and cellular and molecular biology of the *Mx* gene and its products have been studied in mice, rats, and humans (reviewed in reference 20).

Mx has been mapped to MMU 16 (22) and, recently, to HSA 21 (M. Horisberger, M. Wathelet, J. Szpirer, C. Szpirer, Q. Islam, G. Lavan, G. Huez, and J. Content, Somatic Cell Mol. Genet., in press). Six genes and three anonymous DNA segments which map to HSA 21 have been mapped previously to MMU 16, and eight of them have been localized genetically or cytologically to the distal end of the mouse chromosome (1, 15, 17; S. V. Cheng, J. H. Nadeau, R. E. Tanzi, P. C. Watkins, J. Jagadesh, B. A. Taylor, J. L. Haines, N. Sacchi, and J. F. Gusella, Proc. Natl. Acad. Sci. USA, in press). We report here that *Mx* is tightly linked to the proto-oncogene *Ets-2* within the cluster of genes common to the human and mouse chromosomes. Since this region is highly conserved between the two species, it is likely that the human *Mx* gene will be found in the corresponding location on HSA 21.

Molecular probes (6) recognizing the *Mx* gene were synthesized from a 2.3-kilobase *Bam*HI fragment derived from cDNA clone pMx34 (21) or from a 1.7-kilobase fragment derived by *Taq*I digestion of the 2.3-kilobase segment. These probes were used to visualize segregation of *Mx* RFLPs in four backcrosses, two of which have been used previously to localize nine genes on MMU 16. The backcross (Czech II × BALB/cPt) × Czech II is designated CZCxC (15). Czech II is an inbred strain derived from *Mus musculus musculus*. The backcross (CBA/J × BALB/cJ) × BALB/cJ is designated CBCxC (16).

Two additional backcrosses were produced with mice segregating the dwarf (*dw*) gene, (CBA/J × DW/J) × DW/J and (MOLD/Rk × DW/J) × DW/J. These crosses are designated CBDWxDW and MODWxDW, respectively. MOLD/Rk is an inbred strain derived from *Mus musculus molossinus* by Thomas Roderick at the Jackson Laboratory. Male *dw/dw* animals were made fertile by administration of ovine growth hormone (50 μg/day; National Hormone and Pituitary Program, University of Maryland School of Medicine) and thyroxin (2 μg three to five times per week) (Andrej Bartke, personal communication).

DNA was extracted from organs of backcross progeny at 3 weeks of age, when *dw/dw* animals were easily distinguished from *dw/+* littermates by their retarded growth (7). Restriction analysis of *Ets-2* and of the gene encoding the cytoplasmic form of superoxide dismutase, *Sod-1*, was accomplished with molecular probes as described previously (14). Backcross data were compiled in a database with Lotus 1-2-3 software, and a program written in the simple programming language of Lotus 1-2-3 was used to extract different classes of backcross progeny, to determine gene order, and to calculate recombination frequency (14).

RFLPs were used to distinguish *Mx* alleles among the four strains analyzed in this study (Fig. 1). DW/J, BALB/cJ, and BALB/cPt DNAs were identical at all loci. Additional RFLP differences were detected after digestion with *Xba*I, *Hin*cII, *Bam*HI, *Sst*I, and *Taq*I (data not shown). Staehli et al. (21) have shown previously that particular RFLPs are characteristic of strains which are Mx^+ or Mx^-. The restriction patterns obtained from Czech II and MOLD/Rk DNAs with the enzymes *Eco*RI, *Hin*dIII, and *Pst*I are identical to those from DNA of BALB.A2G-Mx mice, which are Mx^+, suggesting that Czech II and MOLD/Rk mice are Mx^+ as well. However, the animals must be challenged with virus to determine with certainty the nature of their Mx phenotypes,

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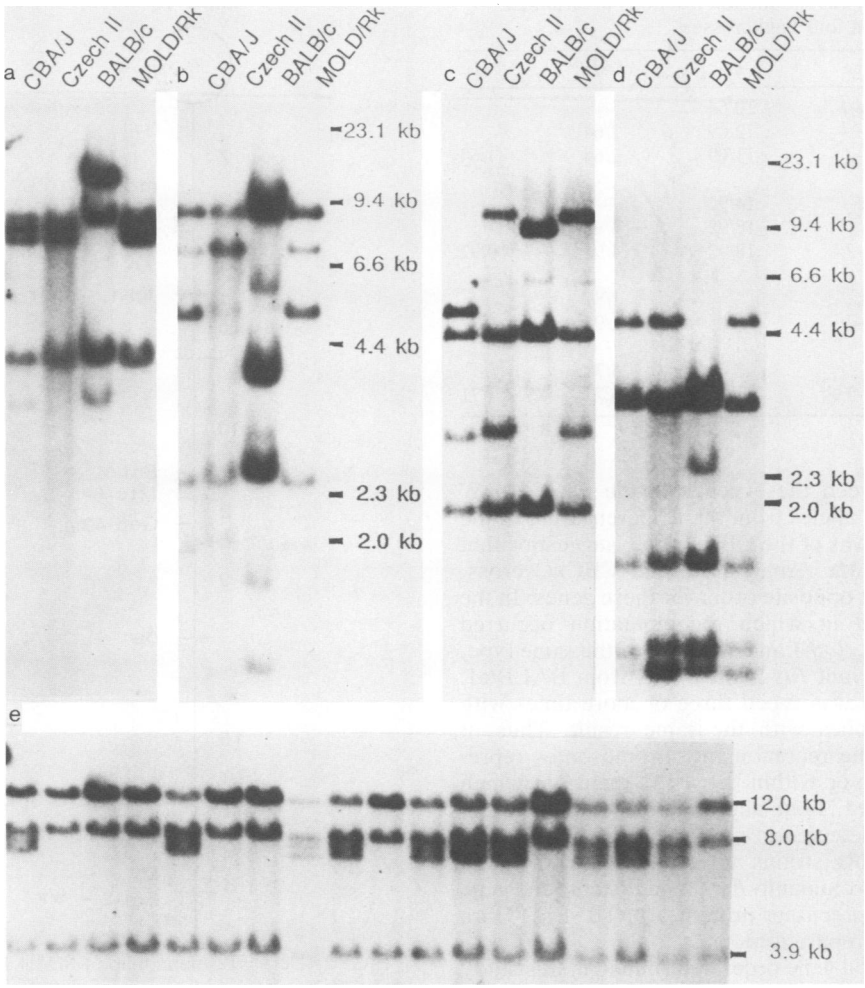

FIG. 1. Restriction analysis of DNAs from strains CBA/J, Czech II, BALB/cJ, and MOLD/Rk with the Mx probe. DW/J and BALB/cJ DNAs gave identical patterns with all restriction endonucleases. The Czech II and MOLD/Rk patterns are identical to those reported for Mx^+ mice (18). (a) *Bgl*II; (b) *Eco*RI; (c) *Hin*dIII; (d) *Pst*I. (e) DNAs from the progeny of the CBCxC backcross analyzed after digestion with *Bgl*II. Positions of molecular weight markers are indicated. kb, Kilobases.

since restriction patterns of DNA from the Mx^- strain, CBA/J, are the same as those seen in DNA from Mx^+ animals with several restriction endonucleases (19a).

In a previous analysis of the CZCxCZ backcross, the *Sod-1* locus was reported to be proximal to *Ets-2* (15). This conclusion was based on a single recombination between these genes among 86 progeny in a three-point analysis with *Mtv-6*, an endogenous retroviral sequence which maps 25 centimorgans (cM) proximal to *Sod-1*. This gene order was confirmed by the analysis of *Sod-1* and *Ets-2* relative to *dw* in the MODWxDW backcross (Table 1). Four recombinations occurred between *Sod-1* and *Ets-2*. In each case, the *Sod-1* allele was of the same parental type as *dw*. An additional recombinant in the CBDWxDW cross was also consistent with this gene order. The map distances calculated on MODWxDW place *Sod-1* 4 cM proximal to *Ets-2* and 15 cM distal to *dw*.

Analysis of *Mx* in 318 animals from the four backcrosses identified only two recombinations between *Mx* and the closest marker, *Ets-2* (Table 2). Thus, these genes are tightly linked, and *Mx* must be near the distal end of MMU 16. While *Mx* is clearly distal to *Sod-1*, its position relative to *Ets-2* cannot be ascertained from these data. In the

TABLE 1. Three-point analysis of *dw*, *Sod-1*, and *Ets-2* on the MODWxDW backcross[a]

dw	*Sod-1*	*Ets-2*	Frequency
M[b]	M	M	33
T[c]	T	T	41
M	M	T	2
T	T	M	2
M	T	T	7
T	M	M	7
M	T	M	0
T	M	T	0

[a] Recombination frequencies: *dw* to *Sod-1*, 0.152 ± 0.037; *dw* to *Ets-2*, 0.196 ± 0.041; *Sod-1* to *Ets-2*, 0.043 ± 0.021. Pairwise recombination frequencies are listed with standard errors. Recombination frequency is the number of recombinations between a gene pair divided by the total number of animals tested (n). Standard error is calculated as $\pm p \times [(1 - p)/n]$, where p is the recombination frequency.

[b] M, Homozygous for the DW/J type allele.

[c] T, Heterozygous for the DW/J type allele.

TABLE 2. Pairwise recombination fractions determined on four backcrosses

Backcross	Gene	*dw*	*Sod-1*	*Mx*
CBDWxDW	*Sod-1*	10/64		
	Mx	12/69	2/64	
	Ets-2	11/69	2/64	1/64
MODWxDW	*Sod-1*	14/92		
	Mx	18/92	4/92	
	Ets-2	18/92	4/92	0/92
CZCxCZ	*Mx*		1/87	
	Ets-2		1/93	0/87
CBCxC	*Mx*		5/71	
	Ets-2		6/71	1/71

single crossover between these genes in the CBDWxDW cross, *Ets-2* and *Sod-1* alleles from the F_1 parent were of the DW/J type, while *Mx* was of the CBA/J type, suggesting that *Ets-2* is proximal to *Mx*. Analysis of the CBCxC cross, however, indicated the opposite order for these genes. In the single CBCxC animal in which recombination occurred between *Ets-2* and *Mx*, *Sod-1* and *Mx* were of the same type, CBA/J, while the relevant *Ets-2* allele was from BALB/cJ. Each of these animals was typed three or more times with each of the three probes with the same result. Thus, it appears that one of the recombinant chromosomes represents a double crossover within the 4-cM region in which these genes are located. Four additional recombinants between *Mx* and *Ets-2* detected in a large backcross with the BALB/cJ and MOLD/Rk strains are consistent with a gene order in which *Mx* is proximal to *Ets-2* and distal to the gene encoding the amyloid precursor protein, *App* (B. F. O'Hara et al., manuscript in preparation).

The proximal-to-distal gene order determined in this study was *dw-Sod-1-(Mx-Ets-2)* (Fig. 2). The *Mx* gene was reported previously to be unlinked to the proximal MMU 16 marker, *md* (55% recombination, $n = 108$) (22), consistent with the results presented here. *Mx* is near several genes which, in humans, are found in the region of HSA 21 associated with Down syndrome when present in three copies. *Sod-1*, *Ets-2*, *App*, and the gene *Prgs*, which encodes the purine synthesis enzyme phosphoribosyl glycinamide synthetase, are located in the region C3 → ter on MMU 16 (1, 12, 15) and on HSA 21 (13, 18, 24). Thus, it is likely that the human *Mx* homolog will be found in the analogous region of HSA 21.

Gene dosage imbalance occasioned by trisomy generally results in an increase in the amount of gene product in the cell. In the case of *IFREC*, the 50% increase in receptor seen in fibroblasts from individuals with Down syndrome results in a three- to eightfold increase in sensitivity to interferon, as measured in a vesicular stomatitis virus protection assay (23). Since *Mx* expression is tightly regulated by interferon, it will be of interest to study *Mx* expression in individuals with Down syndrome, in whom both *IFREC* and *Mx* are present in three copies. Individuals with Down syndrome exhibit impaired immune function including increased susceptibility to opportunistic infections (5).

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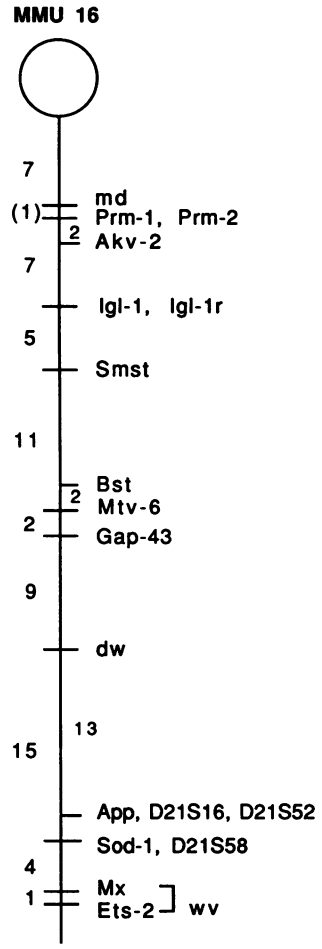

FIG. 2. Derived genetic map of MMU 16. Genes mapped in this study are positioned by averaging data from different crosses. This type of map serves as a basis for comparison of actual distances determined on each cross, which can be determined from Table 2. Positions of markers not examined in this cross are summarized elsewhere (3, 4, 15; Cheng et al., in press; R. H. Reeves, R. A. Morgan, C. Bendotti, M. L. Oster-Granite, J. T. Coyle, and J. D. Gearhart, *in* P. Davies, and C. Finch, ed., *The Molecular Biology of Alzheimer's Disease*, in press; O'Hara et al., in preparation).

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