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Murine Retroviral Restriction Genes *Fv-4* and *Akvr-1* Are Alleles of a Single Locus

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The two murine retroviral restriction genes, *Fv-4* and *Akvr-1*, are very similar in their effects, distributions, ranges of action, and phenotypes. *Akvr-1* has been shown to segregate independently in backcrosses with a variety of retroviral restriction loci, including *Fv-1*, *Fv-2*, *Ril-1*, and *Ril-2*. An allelism test cross of FRG ($Fv\text{-}4^R$) × LC^{RR} ($Akvr\text{-}1^R$) hybrids mated to AKR mice failed to produce any viremic offspring. These results suggested that $Akvr\text{-}1^R$ and $Fv\text{-}4^R$ are alleles of a single locus, *Fv-4*, on mouse chromosome 12.

In laboratory and feral mice, a number of genetic loci have been discovered which specifically delimit the replication and associated pathology of endogenous retroviruses (1, 5, 9, 12, 13). Two of these cellular restriction genes, *Akvr-1* and *Fv-4*, share a number of phenotypic characters, although they were originally discovered in different subspecies of *Mus musculus* (*Akvr-1* in a California population of *M. musculus* subsp. *domesticus* and *Fv-4* from a Japanese strain, FRG, of *M. musculus* subsp. *molossinus*) (1, 3, 8, 10, 13, 14, 16, 17). Both loci exert a strong restriction of ecotropic murine retrovirus in vivo and in vitro. Restriction is dominant, and F_1 hybrids between AKR and $Fv\text{-}4^R$ or $Akvr\text{-}1^R$ are totally free of AKR viremia and the associated neoplastic disease characteristic of the AKR inbred strain (1, 8). In addition, both genes confer dominant resistance to leukemia, lymphoma, and splenomegaly which is induced by exogenous inoculation of newborn mice with NB-tropic Friend murine leukemia virus and NB-tropic Moloney murine leukemia virus. Both loci are polymorphic in modern feral mouse populations and both fail to restrict replication of amphotropic murine leukemia virus. Because of the phenotypic similarities of *Akvr-1* and *Fv-4*, the possibility that the same cellular gene was responsible for both was investigated.

A strain of mice, LC^{RR}, homozygous for the restriction allele of *Akvr-1* has been derived. $Akvr\text{-}1^R$ dominantly restricts ecotropic viremia and leukemia in AKR × LC^{RR} hybrid mice and has been shown previously to segregate in backcross progeny as a single genetic locus (1). LC^{RR} mice and AKR mice differ in coat color genes and electrophoretically at a number of allelic isozymes previously mapped to individual chromosomal loci (11). We determined the recombination frequency between the *Akvr-1* locus and eight such loci on different chromosomes of (AKR × LC^{RR}) × AKR backcross progeny (Table 1). Sera were obtained from parental, F_1, and backcross progeny at 6 to 8 weeks of age and assayed for infectious murine leukemia virus on wild mouse SC-1 cells by using an immunofluorescence assay as described previously (1, 4). Fifty percent of the progeny of this cross were viremic due to the expression of AKR endogenous ecotropic virogene loci and the presence of the $Akvr\text{-}1^s/Akvr\text{-}1^s$ genotype. The remaining 50% were $Akvr\text{-}1^s/Akvr\text{-}1^R$ and thus virus negative. The observed virus restriction was due to the influence of *Akvr-1* and not other restriction loci since AKR and LC^{RR} are isogenic at both *Fv-1* and *Fv-2* for a permissive genotype (Table 2). No linkage between *Akvr-1* and each of these loci, which included genes closely linked to *Fv-1* (*Gpd-1*) and *Fv-2* (*Mod-1*), was detected.

The *Fv-4* locus has been chromosomally mapped to murine chromosome 12 and found to be linked to *Pre-1* and to *Igh-1* by using *Fv-4* alleles derived from both the FRG inbred strain and an outbred wild mouse allele, *Fv-4w* (2, 10, 15). We tested for allelism of *Akvr-1* and *Fv-4* by crossing LC^{RR} mice and FRG mice. LC^{RR} and FRG mice are also isogenic for the permissive alleles of *Fv-1* and *Fv-2* (Table 2). The F_1 animals were crossed to AKR mice to test for independent assortment of *Fv-4* versus *Akvr-1*, using suppression of AKR viremia as our assay for restriction. The results of virus assay of crosses involving these strains are summarized

TABLE 1. Segregation of *Akvr-1*^{R} in (AKR × LC^{RR}) × AKR backcross progeny

Locus[a]	Chromosome	Genotype		No. of mice	% Recombination gene versus *Akvr-1*	χ^b	Retrovirus loci on same chromosome (5–7)
		AKR	LC				
Id-1	1	b	a,c	70	51.4	0.06	*Bxv-1, Ril-2, Mtv-7, 10*
a	2	a	A	42	43.7	0.1	*Rec-1, Ril-1*
Gpd-1	4	b	a,b	68	51.5	0.06	*Fv-1*; end.MMTV, *Ril-3, DBA-CSA*
c	7	c	C	87	52.4	0.02	*Akv-1*; *Fgv-1, Gv-2, Mtv-1*
Gr-1	8	a	a,b	52	48.1	0.08	*Ram-1, Bv, C58v-1*
Mod-1	9	b	a,b	48	60.4	2.1	*Fv-2, Dbv, Sev-1*
Es-3	11	c	a,c	70	57.1	1.42	*Mtv-3, Bbv*
Got-1	19	a	a,b	42	57.1	0.86	

[a] Isozymes were typed by electrophoresis (11) and viremia by the fluorescent-antibody test on SC-1 cells.
[b] χ^2 with 1 df = 3.841 (P = 0.5).

TABLE 2. Mouse strains used in this analysis[a]

Strain	Genotype at restriction locus:				Source
	Fv-1	*Fv-2*	*Fv-4*	*Akvr-1*	
AKR	*N*	*S*	*s*	*s*	Jackson Laboratories
LC	*N*	*S*		*R*	Lake Casitas, M. B. Gardner
FRG	*N*	*S*	*R*		M. Tanigawa, S. Suzuki
AKR (*Fv-1*^{B})[b]	*B*	*S*	*s*	*s*	E. A. Boyse

[a] Genotypes determined in references 1, 10, and 12.
[b] Congenic inbred mouse with *Fv-1*^{B} on an AKR genetic background.

TABLE 3. Infectious murine leukemia virus in progeny of crosses involving *Akvr-1* and *Fv-4*

Cross no.	Maternal	Paternal	No. of litters	No. viremic/ no. tested	Frequency (%)
1	AKR	AKR	7	21/21	100
2	LC^{RR}	LC^{RR}	10	0/42	0
3	AKR	LC^{RR}	5	0/32	0
4[a]	FRG	AKR	ND[b]	0/23	0
5	FRG	LC^{RR}	3	0/20	0
6	AKR	AKR × LC^{RR}	5	19/41	46
7[a]	AKR	AKR × FRG	ND	31/72	43
8	AKR	AKR (*Fv-1*^{B}) × LC^{RR}	5	4/25	16
9	AKR	FRG × LC^{RR}	5	0/33	0

[a] From Odaka et al. (8).
[b] ND, Not determined.

in Table 3. Crosses 1 through 5 demonstrate that both *Fv-4* and *Akvr-1* dominantly suppressed AKR-associated viremia and crosses 6 and 7 demonstrated the 1:1 backcross ratio characteristic of a single-gene locus. Cross 8 provided evidence for independent assortment of *Fv-1* versus *Akvr-1*, indicating lack of allelism for the two restriction genes. Cross 9 demonstrated that *Akvr-1*^{R} and *Fv-4*^{R} segregate from each other since no viremic progeny were obtained from 33 progeny. If the two genes were separate genes located on separate chromosomes, 25% of the offspring would be expected to be viremic. The significant departure from the expectation (χ^2 = 11.1; $P < 0.001$) strongly suggests that the genes are either two rather tightly linked genes or that they are alleles of a single locus. The physiological and phenotypic parallels in gene action (discussed above) of the two genes strongly support the latter interpretation. Within the limits of these reservations, it is suggested that until evidence to the contrary demonstrates duality of these genes they be considered as members of an allelic series of a single locus, *Fv-4*.

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