マウスにおける突然変異gracile axonal dystrophy(gad)遺伝子座の位置決定(第5染色体)

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Location of gracile axonal dystrophy (*gad*) on chromosome 5 of the mouse

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ABSTRACT

Linkage tests of gracile axonal dystrophy (*gad*), an autosomal neurological mutation in the mouse, have shown that *gad* was located on chromosome 5 and linked to *Pgm-1* and *W*. On the basis of the backcross data, the following recombination frequencies were calculated: *Pgm-1-gad*, 3.0±1.0 percent; *W-gad*, 5.4±2.6 percent. Therefore, the gene order was estimated to be centromere-*gad-Pgm-1-W*. Since *Pgm-1* was closely linked to *gad*, *Pgm-1* is a useful marker to recognize homozygotes (*gad*/*gad*) before onset of clinical signs and detect carriers (*gad*/+).

1. INTRODUCTION

The autosomal recessive mutation, gracile axonal dystrophy(*gad*) caused degeneration in the gracile nucleus of medulla oblongata and gracile fascicules of spinal cord characterized by neuroaxonal dystrophy or focally swollen axons. Homozygous mice developed spinal ataxia following a normal growth until about 80 days of age. But they could be distinguished from normal mice by the abnormal position of the hind limbs when the animal was hung by the tail after 1 month of age. In view of pathological findings, gracile axonal dystrophic mice could be a useful animal model for neuroaxonal dystrophy occurring in infantile neuroaxonal dystrophy and Hallervorden-Spatz disease of humans, vitamin E deficiency and aging (Yamazaki *et al.* 1988).

This paper reports linkage of *gad* with *Pgm-1* and *W*, and the gene order of these three loci on chromosome 5 of the mouse.

2. MATERIALS AND METHODS

Mice

The strains used were Gad/Nga, SM/J, SK/Cam, C57BL/6, NZB and WN

Tabel 1. *Distribution of morphological and biochemical markers in the strains used for linkage tests*

Chromosome No.	1	2	3	4	5	5	6	6	7	8	14
Marker loci	*Idh-1*	*a*	*Car-2*	*Pgm-2*	*Pgm-1*	*W*	*Ldr-1*	*Ggc*	*Hbb*	*Es-1*	*Es-10*
Strains											
Gad/Nga	b	a	a	a	a	+	a	a	d	b	b
SM/J	b	a	b	b	a	+	a	a	s	b	b
SK/Cam	a	A	a	a	a	+	b	b	d	b	b
C57BL/6	a	a	a	a	a	+	a	b	s	a	a
NZB	a	a	a	a	b	+	a	a	d	b	b
WN	a	a			a	W^n			s	a	

(Staats 1980; Go *et al.* 1980) which were maintained by full-sib matings in the Laboratory of Animal Genetics, Faculty of Agriculture, Nagoya University, Japan.

Linkage tests

Linkage tests were carried out using backcross offspring and chi-square tests were calculated for the *gad* and marker locus segregation data based on an expected 1 : 1 segregation ratio.

Marker gene loci used were as follows: agouti (*a*), carbonic anhydrase-2 (*Car-2*), dominant spotting (*W*), esterase-1 (*Es-1*), esterase-10 (*Es-10*), γ-glutamyl cyclotransferase (*Ggc*), hemoglobin β-chain (*Hbb*), isocitrate dehydrogenase-1 (*Idh-1*), lactate dehydrogenase regulator-1 (*Ldr-1*), phosphoglucomutase-1 (*Pgm-1*) and phosphoglucomutase-2 (*Pgm-2*). Distribution of these markers in the strains used are shown in Table 1.

Blood samples were collected with heparinized capillary tubes from the orbital sinus, and centrifuged at 3,000 rpm for 15 minutes to obtain plasma and erythrocyte frations. Packed red blood cells of erythrocyte fraction were washed three times by repeating centrifugation in saline. The mice were sacrificed by cervical dislocation, kidneys were rapidly removed and homogenized in a fivefold volume of distilled water at 0°C. The homogenates were centrifuged at 15,000 rpm at 0°C for 30 minutes to obtain supernatants. The samples were immediately subjected to electrophoresis or stored at −20°C prior to use.

Titan III electrophoresis (Herena Laboratory, U.S.A.) was carried out to type *Car-2, Es-1,* Es-*10, Hbb, Idh-1, Ldr-1, Pgm-1* and *Pgm-2* (Nomura *et al.* 1984). For typing of *Ggc,* starch gel electrophoresis was performed after the method of Orlowski and Meister (1973) except that horizontal electrophoresis was substituted for vertical one (Tulchin and Taylor 1981).

3. RESULTS AND DISCUSSION

The data presented here show that *gad* is not linked to *Idh-1, a, Car-2, Pgm-2, Ldr-1, Ggc, Hbb, Es-1* and *Es-10* (Table 2), but linked to *Pgm-1* with a recombination frequency of 3.0±1.0 percent (Table 3), and to *W* with a recombination frequency of 5.4±2.6 percent on chromosome 5 (Table 4). This is not inconsistent with the estimate of recombination between *Pgm-1* and *W* to be 3.0±1.7 percent (Hutton and Roderick 1970). Thus the gene order is estimated to be centromere-*gad-Pgm-1-W*. However, three point cross would be necessary to define precise distances between three loci and their order, since they are located very closely one another.

Pgm-1 is closely linked to *gad* and the genotype of it can be determined for individual mice without killing. Therefore, *Pgm-1* can be a useful marker

Table 2. *Results of linkage tests with gad*

Marker loci	Non-recombinants Pooled (*gad*/*gad* *gad*/+)	Recombinants Pooled (*gad*/*gad* *gad*/+)	Total	χ^2	
Idh-1	35 (13 22)	36 (16 20)	71	0.0141	$0.90<P<0.95$
a	27 (10 17)	31 (14 17)	58	0.276	$0.50<P<0.70$
Car-2	24 (12 12)	25 (9 16)	49	0.0204	$0.90<P<0.95$
Pgm-2	24 (8 16)	25 (13 12)	49	0.0204	$0.90<P<0.95$
Ldr-1	35 (17 18)	36 (12 24)	71	0.0141	$0.90<P<0.95$
Ggc	38 (18 20)	33 (11 22)	71	0.352	$0.50<P<0.70$
Hbb	50 (28 22)	57 (26 31)	107	0.458	$0.30<P<0.50$
Es-1	43 (25 18)	50 (25 25)	93	0.527	$0.30<P<0.50$
Es-10	50 (25 25)	43 (25 18)	93	0.527	$0.30<P<0.50$

Table 3. *Linkage analysis between GAD and PGM-1*[1]

Mating	Genotypes of progeny			
	Non-recombinants		Recombinants	
	gad Pgm-1^a	+ *Pgm-1*^{b}	*gad Pgm-1*^{b}	+ *Pgm-1*^{a}
	gad Pgm-1^a	*gad Pgm-1*^{a}	*gad Pgm-1*^{a}	*gad Pgm-1*^{a}
gad Pgm-1^a + *Pgm-1*^{b} / *gad Pgm-1*^{a} *gad Pgm-1*^{a}	147	147	4	5
Pooled progeny	294		9	

[1] Recombination frequency=3.0±1.0%; χ^2=269.1; df=1.

gene to distinguish affected mice from normal ones to examine physiological and pathological changes before one month of age, when clinical distinction is impossible. Moreover, distinction between +/+ and *gad*/+mice is possible.

Linkage of *Pgm-1*, *Pep-7* (peptidase-7), *Alb-1* (serum albumin variant) and *Afp* (α-fetoprotein) on mouse chromosome 5 is conserved on human chromosome 4 (Lalley and McKusick 1985). Murine *Pgm-1*, *Pep-7*, *Alb-1* and *Afp* are homologous to human *PGM2*, *PEPS* *ALB* and *AFP*, respectively. Since linkage of *Pgm-1* and *gad* is very close in mice, it is possible that human disease locus homologous to *gad* is linked to *PGM2*. At present no potential

Table 4. *Linkage analysis between GAD and W*[1]

Mating				Genotypes of progeny							
				Non-recombinants				Recombinants			
				gad	+	+	W^n	*gad*	W^n	+	+
				gad	+	*gad*	+	*gad*	+	*gad*	+
gad	+	*gad*	+								
———	×	———		27		43		1		3	
gad	+	+	W^n								
Pooled progeny				70				4			

[1] Recombination frequency=5.4±2.6%; χ^2=58.9; df=1.

human homologue linked to *PGM2* has been identified.

There are some clinical and pathological differences between gracile axonal dystrophy in mice and the two human diseases, namely infantile neuraxonal dystrophy and Hallervorden-Spatz disease. But both of them are characterized pathologically by the presence of spheroids or neuroaxonal dystrophy and considered to be inherited as an autosomal recessive trait (Seitelberger 1971; Elejalde *et al.* 1979). These human diseases have not been mapped so far (Wisniewski *et al.* 1985). Thus it is of interest to examine linkage of *PGM2* with these disease loci.

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