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Chromosomal Mapping of Genetic Locus Associated with Thymus-size Enlargement in BUF/Mna Rats

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The thymoma-prone rat of the BUF/Mna strain is a useful model for human thymoma. In this strain thymoma development is regulated by a single autosomal susceptible gene, *Tsr-1*. At pre-thymoma age, BUF/Mna rats have extremely large thymuses, when compared to those of other strains of rats. Genetic studies in crosses between BUF/Mna rats with large thymuses and WKY/NCrj rats with small thymuses suggested the presence of a major autosomal gene, *Ten-1*, which contributes to thymus enlargement in a backcross population. Linkage studies between *Ten-1* and microsatellite markers in backcross rats of (WKY/NCrj × BUF/Mna)F1 × BUF/Mna have led to the localization of *Ten-1* in chromosome 1. This result may provide an approach to clone *Tsr-1*, which could be allelic to *Ten-1*.

Key words: *Ten-1* — Large thymus — Chromosome 1 — BUF/Mna rat — *Tsr-1*

Slight differences in thymus weight among inbred rat strains have been observed. In the course of a study on the development of thymoma, we noticed that the thymuses of BUF/Mna rats were much larger in suckling, young adult and adult periods than those of ACI/NMs rats.[1)] Genetic studies in crosses between BUF/Mna and WKY/NCrj rats revealed a single major locus, *Ten-1*, which is associated with thymus enlargement.[2)] Hybrid and backcross rats between the BUF/Mna strain and WKY/NCrj, ACI/NMs, F344 or BDIX strain having a larger thymus ratio than 5.3 at 6 weeks of age developed thymoma in old age (unpublished data), suggesting that *Tsr-1* could be allelic to *Ten-1*. These findings prompted us to perform molecular studies to examine the localization of *Ten-1*.

In order to map the *Ten-1* gene, {(WKY/NCrj × BUF/Mna)F1 × BUF/Mna} backcross rats were obtained by matings. They were killed at 6 weeks of age, and the thymuses were removed and weighed. Spleens were also removed for extraction of genomic DNAs.

Microsatellite sites of each DNA (25–50 ng) were amplified by a slightly modified polymerase chain reaction (PCR) method described previously,[3)] gel-electrophoresed and stained with ethidium bromide. We selected 42 microsatellite markers that showed length variations of the PCR product among 8 inbred rat strains.[3)] Ten of these 42 markers showed length polymorphism between the BUF/Mna (B) and WKY/NCrj (W) strains (Table I). One of the 10 markers, *MYL2* which resides in chromosome 1, had linkage to the thymus ratios (thymus weight/body weight; mg/g) (Figs. 1 and 2, Table I). The *MYL2* marker detected 104-bp and 122-bp bands in DNAs of BUF/Mna rats and 90-bp and 102-bp bands in those of WKY/NCrj rats (Fig. 1, lanes 1 and 3). Since this marker always produced two bands under our experimental conditions, it may be due to the presence of two binding sites of the primer in the *MYL2* locus. The backcross rats were classified into 2 groups according to the thymus ratios: higher or lower than the median value of 5.3. DNAs from 25 of 36 rats (69%) with higher thymus ratios contained the 104-bp and 122-bp bands, indicating that they were B/B homozygous at the *MYL2* marker (Fig. 1, lanes 4, 5 and 8, and Table I). On the other hand, DNAs from 23 of 32 rats (72%) with lower thymus ratios were B/W heterozygous at this marker (Fig. 1, lanes 6, 7 and 9, and Table I). Thus, rats with higher thymus ratio were more often homozygous at *MYL2* locus than rats with smaller thymus ratio ($\chi^2=11.8$; $P<0.001$; Table I). Another marker, *KAL*, which also resides in chromosome 1, showed no linkage to the thymus size ($\chi^2=0.8$; Table I). Furthermore, the other 8 markers, *MT1PB, CPB, SVS2P, ENO2, PND, AEP, SYB2* and *ACRM* which reside in other chromosomes showed no linkage to the thymus ratios. These results reveal that *Ten-1* resides in chromo-

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Table I. Associations of Microsatellite Markers with Thymus Size in {(WKY/NCrj×BUF/Mna)F1×BUF/Mna} Backcross Rats

Microsatellite marker	Nos. of backcross rats				Value of χ^2-test
	With large thymus[a]		With small thymus[a]		
	Homozygous (B/B)	Heterozygous (B/W)	Homozygous (B/B)	Heterozygous (B/W)	
KAL	20	16	15	17	0.8
MYL2	25	11	9	23	11.8
MT1PB	15	21	18	14	1.8
CPB	17	19	21	11	3.3
SVS2P	19	17	17	15	0.5
ENO2	19	17	17	15	0.5
PND	16	20	12	20	2.6
AEP	20	16	16	16	0.7
SYB2	24	12	15	17	4.6
ACRM	12	24	17	15	4.6

a) Sixty-eight backcross rats were classified into 2 groups according to thymus ratio (thymus weight/body weight; mg/g); higher or lower than the median value of 5.3. Six rats with thymus ratios of 5.3 were not included.

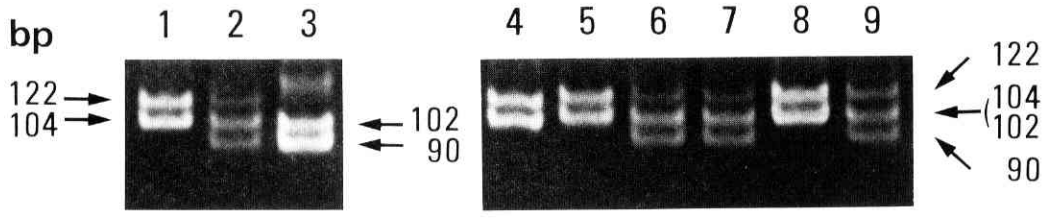

Fig. 1. PCR analysis of *MYL2* markers in BUF/Mna (lane 1), (WKY/NCrj×BUF/Mna)F1 (lane 2), WKY/NCrj (lane 3) and {(WKY/NCrj×BUF/Mna)F1×BUF/Mna} backcross rats (lanes 4–9). Lanes 4, 5 and 8: B/B homozygote; lanes 6, 7 and 9: B/W heterozygote.

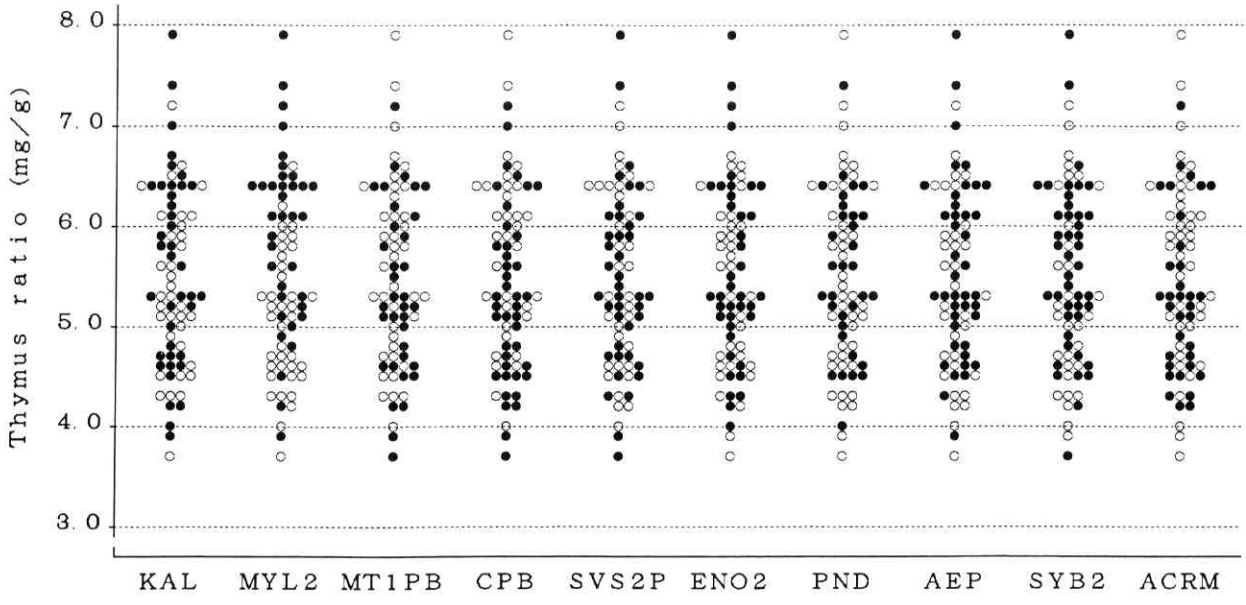

Fig. 2. Thymus ratios and genotypes of *KAL, MYL2, MT1PB, CPB, SVS2P, ENO2, PND, AEP, SYB2* and *ACRM* of {(WKY/NCrj×BUF/Mna)F1×BUF/Mna} backcross rats. ●: B/B homozygote, ○: B/W heterozygote.

some 1. The finding that there was no linkage between thymus size and *KAL* might be due to the distance between *Ten-1* and *KAL*. It is known that *KAL* belongs to the classical linkage group II, whereas *MYL2* belongs to the classical linkage group I in the rat, having a large recombination fraction (0.58) between these 2 loci.[3,4]

In the mouse, *Tsz-1* (thymus size 1) locus was reported to control the thymus size in young adult and adult periods.[5] Remarkable increases in thymus size have also been reported in mice bearing an inheritable defect in the androgen protein (testicular feminization: Tfm/Y).[6] Our results demonstrate that the gene for thymus enlargement resides in chromosome 1 in the BUF/Mna rat, although it is still possible that genes located in other loci are also involved.

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