*Exp. Anim. 49(1), 43–45, 2000*

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# *Mapping of the beige (bg) Gene on Rat Chromosome 17*

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***Abstract:*** *The rat beige (bg) autosomal recessive gene, causing Chediak-Higashi Syndrome (CHS) in rat, was mapped on Chr 17 by using synteny of rat to mouse and humans. The linkage between the beige gene and PCR-amplified microsatellite markers in (DA-bg × BN)$F_1$ × DA-bg backcross progeny was analysed. The recombination frequency was 9.5% between Prl and Acrm and 19.1% between Acrm and bg. The proposed order of three genes is Prl-Acrm-bg. This rat bg gene was confirmed to be homologus to the beige (bg) gene of mouse located on Chr 13 and the CHS (Lyst) gene of man located on Chr 1 (1q43).*

***Key words:*** *Beige rat, Chediak-Higashi Syndrome of rat, gene mapping*

Chediak-Higashi Syndrome (CHS) is an autosomal recessive genetic disease that has been reported in man [1, 2], killer whales [15], mink [4], cattle [16], and mice [5]. CHS was first recognized as a disorder in which melanocytes, neutrophils and lymphocytes contained giant cytoplasmic granules. A mutation comparative to human CHS has been found in mice which have a characteristic diluted coat colour, and named "beige". A counterpart in rats has been awaited for studies, such as biochemical, immunological and histological analysis of CHS in humans. In 1989 we found that the coat colour mutant DA/Ham rat maintained in Hamamatsu University School of Medicine was comparable to human CHS and the strain was named DA-*bg* (beige rat) [9]. Pathological [11, 12], parasitological [3], molecular genetical [8] and hematological [10] studies have been performed on this rat, but no genetical analysis could be performed, because of the lack of practical linkage markers.

In the present study, we report a genetic linkage map of the *bg* gene and two microsatellite markers on rat Chr 17. The linkage map shows conservation of the synteny in particular regions of rat Chr 17, mouse Chr 13 and human Chr 1q43.

To examine the linkage between beige coat colour and microsatellite markers, we backcrossed (DA-*bg* × BN/Ham) $F_1$ to DA-*bg*. Of 167 offspring obtained, 73 had a beige coat colour and 94 an agouti coat colour (Fig. 1).

Two sets of primers, R103 and R23 were designed to define $(AC)_{18}$ microsatellite in the *Acrm* (Acetylcholine receptor, m3 muscarinic) locus and $(TG)_{15}$ microsatellite in the *Prl* (Prolactin) locus on Chr 17 respectively [13]. DNA was extracted from the liver and dissolved in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. PCR was performed with a type PTC-100 Programmable Ther-

*(Received 25 March 1999 / Accepted 8 July 1999)*

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**Fig. 1.** Coat colour of DA-+/+ (agouti), behind and DA-*bg/bg* (beige), in front. $F_1$ had the colour of DA-+/+ (agouti).

mal Controller (MJ Research, Inc. Watertown, MA, USA). The reaction volume was 50 $\mu l$ containing 50 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% w/v gelatin, 200 $\mu$M dNTPs, 1 unit of Taq DNA polymerase (Perkin Elmer, Norwalk, CT, USA) and 10 pmol/$\mu l$ of each primer. Temperature and time cycles were: 3 min at 94°C, 35 cycles of 1 min at 94°C 1 min at 55°C and 30 sec at 72°C, followed by a final 10 min elongation step at 72°C. PCR products were fractionated by 4% agarose gel electrophoresis (Nusieve:SeaKem 3:1 agarose, FMC BioProduct, Rockland, ME, USA) and stained by ethidium bromide.

Linkage analysis was performed as follows. Both of the microsatellite markers, *Acrm* and *Prl* loci were polymorphic in the parental strains, and the coat colour and the genotype were examined with the two microsatellite markers in all back cross progeny (Table 1). The distance between beige coat colour and two microsatellite markers was calculated from the recombination values derived from the phenotypes and genotypes of the backcross progeny.

From the data shown in Table 1, the recombination values were calculated to be 9.5% ± 2.2% between *Prl* and *Acrm*, 19.1% ± 3.0% between *Acrm* and *bg*, and 28.1% ± 3.4% between *Prl* and *bg*. The proposed order of these three loci is *Prl*-(9.5 cM)-*Acrm*-(19.1 cM)-*bg* on Chr 17.

In the mouse, the gene order on chromosome 13 is centromere-(17 cM)-*bg*-(4 cM)-*Prl* [7]. The rat Chr 17 is homologus to the mouse Chr 13 [14, 17] and the gene order we obtained was *bg*-(28 cM)-*Prl*. To determine the linkage with other markers on Chr 17, we examined a total of 14 microsatellite markers on rat Chr 17 and 7 microsatellite markers on mouse Chr 13. All of these microsatellite markers were purchased from Research Genetics Inc. Huntsville, AL, USA, but between BN and DA-*bg*, these microsatellite markers were not useful.

Recently, in a molecular study on the Chediak-Higashi syndrome this gene was mapped to Chr 1q43 [6].

According to the genetic linkage map; the rat Chr 17 is homologus to mouse Chr 13 and also to human Chr 1 [17]. In particullar, the 1q43 region of human Chr 1 is highly homologus to the rat Chr 17, which contains the *Acrm* locus. In this study we confirmed that the rat beige (*bg*) gene is located on Chr 17, and shows homology to mouse Chr 13 and human Chr 1 (Fig. 2).

The rat is one of the most common laboratory animals for biomedical research, because of its convenient body size. Rat models are useful for genetic studies of human diseases. Furthermore, a genetic linkage map for comparison with those of mice and man has been reported. The beige rat might be an interesting alternative model for the study of human CHS and to establish a remedy for human CHS.

## Acknowledgments

The authors thank Dr. T. Serikawa of Kyoto University for kindly providing the primers and useful advice.

**Table 1.** Linkage analysis of beige coat colour and two microsatellite markers

| gene symbol | non-recombinant | | recombinant | | $\chi^2$ value | recombination value | S.E. |
|---|---|---|---|---|---|---|---|
| *Prl - Acrm* | 66 | 85 | 10 | 6 | 113.6 | 9.5 | 2.2 |
| *Acrm - bg* | 57 | 75 | 16 | 19 | 69.2 | 19.1 | 3.0 |
| *Prl - bg* | 49 | 71 | 24 | 23 | 37.7 | 28.1 | 3.4 |

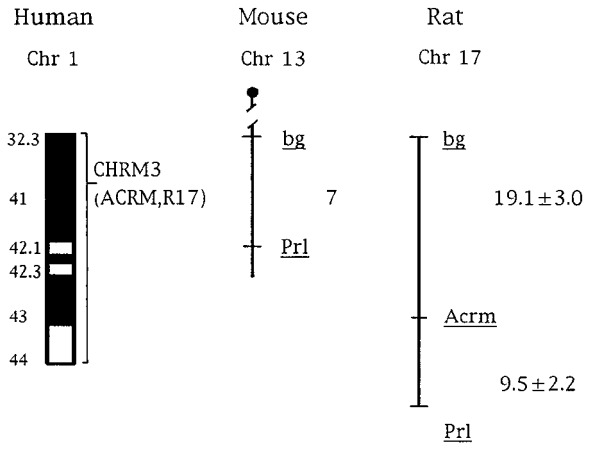


**Fig. 2.** Linkage map of the rat Chr 17, showing the *bg* locus. The comparative maps for the human chromosome 1 and mouse Chr 13 are shown [7, 17]. Numbers to the left of the chromosome indicate estimated distance in cM from the centromere.

This study was supported by Grant in Aid No. 06680828 from the Japanese Ministry of Education, Science, Sports and Culture.

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