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## SHORT PAPER

# The susceptibility to cortisone-induced cleft palate of recombinant inbred strains of mice: lack of association with the *H-2* haplotype

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SUMMARY

Recombinant-inbred (RI) strains of mice derived from the cross of strains C57BL/6J and DBA/2J were used to study the inheritance of susceptibility to cortisone-induced cleft palate. Most of the RI strains could be classified as either resistant, like C57BL/6J, or susceptible, like DBA/2J, suggesting the segregation of a major locus. An association with the phosphoglucomutase-1 locus (*Pgm-1*) on Chromosome 5 was observed. There was no association with the *H-2* locus on Chromosome 17 as had been reported in previous studies utilizing different strains. We conclude that susceptibility to cortisone-induced cleft palate may be determined by different loci depending on the strains studied.

## 1. INTRODUCTION

The genetic basis of cortisone-induced cleft palate has been studied in only a small number of strains of mice, particularly the highly inbred A/J and C57BL/6J strains (Kalter, 1954; Biddle & Fraser, 1976; Biddle & Fraser, 1977*a*). In these two strains the embryonic difference in susceptibility to cortisone-induced cleft palate is determined by at least two 'major' loci, presumably acting independently. One of them appears to be linked to the major histocompatibility-2 locus (*H-2*) on Chromosome 17 in the mouse (Biddle & Fraser, 1976; Biddle & Fraser, 1977*a*). This has been taken to imply some fundamental relationship between the *H-2* locus and the morphogenetic basis of palate closure (Goldman *et al.* 1977). However differences between these two strains represent only a small portion of the total genetic variation (Taylor, 1972) and the genes that cause the difference between the A and C57BL strains are not necessarily the same as those that cause the difference among other strains.

Using the recombinant-inbred strains derived from the cross of strains DBA/2J and C57BL/6J, we have shown an association between susceptibility to cortisone-induced cleft palate and the phosphoglucomutase-1 locus (*Pgm-1*) on Chromosome 5 in the mouse (Shows, Ruddle & Roderick, 1969).

## 2. MATERIALS AND METHODS

Recombinant-inbred strains (RI) have proved to be a valuable tool in immunogenetics and biochemical genetics to demonstrate genetic independence, linkage and pleiotropism (Bailey, 1971; Swank & Bailey, 1973; Taylor, 1978) and their use in experimental teratology should be fruitful (Biddle & Fraser, 1977*b*). They are derived by brother-sister inbreeding, beginning with the F2 generation derived from the cross between two inbred strains (progenitors). Once inbred, the RI strains are typed with respect to the numerous genetic loci at which the progenitor strains have different alleles. The allele from either one or the other parental strain is present in each of the RI strains. Loci that are closely linked will show a non-random association of alleles.

The recombinant-inbred strains derived from a cross between the progenitor strains C57BL/6 and DBA/2 (BXD RI) were developed by one of us (B.A.T.), and transferred to the McGill mouse room for testing. They are outstanding with respect to the number of strains (26), the number of loci at which they have been typed (100) and the number of chromosomes with at least one identified marker (14) (Taylor, 1978 and unpublished data). They have been inbred for at least 20 further generations, so that the likelihood of encountering residual heterozygosity at any particular locus is slight.

The mouse room at McGill University was maintained at 22 °C with 16 h light and 8 h dark periods. The mice were fed Purina Mouse Chow from the time they were received and switched to Purina Laboratory Chow only during pregnancy. They received water *ad libitum*. Females were time-mated and checked for vaginal plugs the following morning. Fertilization was assumed to occur at 2 a.m. the day the plug was found (Snell *et al.* 1940), which was considered to be day 0 of gestation.

On day 12, females were weighed and palpated for confirmation of pregnancy. Cortisone acetate (kindly provided by Merck, Sharp, and Dohme, Montreal) was injected subcutaneously in the interscapular region of pregnant females at noon. Dosage (200 mg/kg) was proportional to weight at time of treatment. At this dose the frequencies of cleft palate with their 95 % limits in the two progenitor strains, C57BL/6J and DBA/2J are 4 % (1–13) and 68 % (49–84) respectively (Vekemans & Fraser, 1980).

On day 17, females were killed by cervical dislocation and the uteri were fixed in Bouin's solution, and examined 1 week later. Foetuses were sexed after analysis of the gonads and examined for cleft palate. All litters with less than three foetuses were excluded from the tabulation of cleft palate response.

## 3. RESULTS AND DISCUSSION

The distribution of cleft palate response appears to be bimodal in the RI strains (Table 1). Thus, a major part of the variation in the trait is presumably controlled by one locus. However, two strains (BXD-24 and BXD-9), show an intermediate response. Since their mean cleft palate frequencies fall outside the confidence limits of the C57BL/6J strain but within those of the DBA/2J strain, they have been tentatively classified as D. However, if this is not simply a result of small sample variation it suggests that other factors influence the difference in response between the two progenitor strains.

The BXD RI strains had been typed previously with respect to numerous genetic markers. Therefore, it was possible to compare the pattern of cleft palate response with the marker patterns determined, to look for association that might suggest either linkage of the putative cleft palate response gene to some other locus, or a pleiotropic effect of some previously described locus on the cleft palate response. These comparisons revealed a significant association ($P < 0{\cdot}01$) between cleft palate response and the phosphoglu-

comutase-1 locus (*Pgm-1*) on Chromosome 5 (Table 2). Only two of the 14 BXD RI strains are discordant. Four of 13 strains were discordant for the cleft palate response and *Ric*, a gene controlling scrub typhus resistance (Groves *et al.* 1980), which is also on Chromosome 5. The pattern of recombinants is consistent with the placement of a major locus controlling cleft palate response between *Pgm-1* and *Ric* on Chromosome 5. These

Table 1. *Cleft palate response in BXD RI strains*

| BXD RI strains | Number of litters | Cleft Palate/Live Foetuses | | |
|---|---|---|---|---|
| | | No. | % | 95% limits* |
| 1 | 2 | 1/11 | 9 | 0–41 |
| 2 | 4 | 31/35 | 89 | 73–97 |
| 5 | 4 | 29/29 | 100 | 88–100 |
| 6 | 4 | 7/33 | 21 | 9–39 |
| 9 | 2 | 7/15 | 47 | 21–73 |
| 11 | 3 | 0/13 | 0 | 0–25 |
| 14 | 3 | 16/16 | 100 | 79–100 |
| 16 | 3 | 23/23 | 100 | 85–100 |
| 18 | 4 | 34/34 | 100 | 90–100 |
| 19 | 3 | 16/18 | 89 | 65–99 |
| 21 | 4 | 26/26 | 100 | 87–100 |
| 24 | 5 | 13/32 | 41 | 24–59 |
| 29 | 6 | 2/46 | 4 | 0–15 |
| 32 | 2 | 7/10 | 70 | 35–93 |

* (Documenta Geigy, 1963).

Table 2. *Inheritance of cleft palate responses and alleles at the* Pgm-1 *and* Ric *loci in 14 BXD RI strains**

| Locus | BXD RI Strain | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 5 | 6 | 9 | 11 | 14 | 16 | 18 | 19 | 21 | 24 | 29 | 32 |
| Cleft palate† | B | D | D | B | D | B | D | D | D | D | D | D | B | D |
| *Pgm-1*‡ | B | D | D | B | D | B | B | D | B | D | D | D | B | D |
| *Ric*‡ | B | D | D | B | B | D | D | D | D | B | D | D | D | – |
| *H-2*§ | D | B | D | D | D | D | B | D | D | B | D | D | B | D |

* The letters B and D in the body of the table are used as generic symbols for alleles inherited from the progenitor strains C57BL/6J and DBA/2J, respectively.

† The classification of RI strains as either C57BL/6J-like (B) or DBA/2J-like (D) is tentative (see Table 1).

‡ The classification of the BXD RI strains with respect to the *Pgm-1* and *Ric* loci is published (Groves *et al.* 1980).

§ The *H-2* typing was provided by Dr Marianna Cherry (personal communication).

results do not prove linkage, however, and a coincidental association has not yet been entirely excluded. There was clearly no association with the *H-2* locus on chromosome 17 (six recombinants among 14 strains).

During the successive generations of brother-sister inbreeding in the formation of RI strains, there are multiple opportunities for recombination between linked loci. The probability of a recombinant genotype becoming fixed ($R$) is $4r/(1+6r)$, where $r$ is the

probability of recombination in a single meiosis (Haldane & Waddington, 1931). This relationship provides a basis for a rough estimation of recombinant frequency from linkage data obtained in RI strains. Assuming that the association with *Pgm-1* is real and the classification of the BXD strains in Table 2 is correct, we can substitute 2/14 for $R$ to obtain an estimate of $r$. The estimated variance of $r$ is given by $r(1+2r)(1+6r)^2/4N$ where N is the number of RI strains. The estimate of $r$ and its standard error obtained in this way is $0{\cdot}05 \pm 0{\cdot}04$. As this is a relatively short distance, we are justified in equating recombination with map distance. Thus the estimated map distance between *Pgm-1* and the locus controlling the DBA-C57BL difference in cleft palate response to cortisone is $5 \pm 4$ centimorgans (cM).

In summary, the strain difference in cortisone reactivity between the DBA/2 and C57BL/6 strains appears to be relatively simple. One major locus, that is linked to *Pgm-1*, contributes most of the difference in reactivity but embryonic response is also influenced by other genetic factors. These findings support the idea that a small number of genes determines the difference between pairs of inbred strains in sensitivity to cortisone-induced cleft palate. However, different genes may be involved in different strains, so that the total number of genes influencing sensitivity may be large.

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