Length of the chromosomal segment marked by galactose-1-phosphate uridyl transferase and soluble aconitase and conserved since divergence of lineages leading to mouse and man

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Summary

Standard linkage testing crosses and ovarian teratoma mapping were used to estimate the length of the chromosomal segment that is marked by galactose-1-phosphate uridyl transferase and soluble aconitase and that has been conserved since divergence of lineages leading to mouse and man. These experiments were also used to determine whether the $Rb(4-6)2Bnr$ Robertsonian translocation suppresses recombination on the proximal portion of mouse Chromosome 4. The estimated length of the conserved segment marked by galactose-1-phosphate uridyl transferase and soluble aconitase in mouse and man was estimated to be 24 cM. It was also shown that $Rb(4-6)2Bnr$ strongly suppressed recombination on the centromeric portion of mouse Chromosome 4.

1. Introduction

Lengths of chromosomal segments that have been conserved since divergence of lineages such as those leading to mouse and man provide valuable information concerning the organization and evolution of the mammalian genome. These lengths can be used to estimate the rate and number of linkage disruptions that have occurred since divergence of lineages and to test whether chromosomal rearrangements that disrupt linkage are randomly distributed in the genome (Nadeau & Taylor, 1984; Nadeau & Reiner, 1988). Accurate estimates of segment length are essential for these analyses. One of these putative preserved autosomal segments is the segment marked by galactose-1-phosphate uridyl transferase ($GalT$), soluble aconitase ($AcO-I$), aminolevulinate dehydratase ($Le$), and orosomucoid-1 ($Orm-I$). These genes are located on mouse Chromosome (Chr) 4 (Nadeau & Eicher, 1982; Baumann, Held & Berger, 1984; Baumann & Berger, 1985; Nadeau et al. 1986). The homologues of $GalT$ and $AcO-I$ are located on chromosome 9p in man, $Orm-I$ is located on chromosome 9q, and although the arm assignment for the homologue of $Le$ has not been confirmed, it is probably 9q (Westerveld et al. 1975; Mohandas et al. 1977, 1979; Bruns et al. 1978; Cook et al. 1978; Aitken & Ferguson-Smith, 1979; Mulcahy & Wilson, 1980; Eiberg, Mohr & Staub-Nielsen, 1982, 1983). There is uncertainty about the length of the segment marked by $AcO-I$ and $GalT$ in the mouse, however, because the cross used to measure the recombination frequency involved a Robertsonian translocation $Rb(4-6)2Bnr$ (hereafter abbreviated $Rb2$) (Nadeau & Eicher, 1982). It is well-established that Robertsonian translocations suppress recombination on the centromeric portions of certain chromosomes (Cattanach, 1978). Recombination suppression imposed by $Rb2$ is controversial, however, because one of the genes used in the original study (Cattanach, 1978), $asp$, is no longer believed to be located on Chr 4 (Seyfried & Glaser, 1981). The purpose of this note is to estimate the length of the segment marked by $GalT$ and $AcO-I$ and to determine whether the $Rb2$ translocation suppresses recombination on the proximal portion of Chr 4.

2. Materials and methods

(i) Mice

Mice were obtained from the research and production colonies of the Jackson Laboratory.

(ii) $GalT$ and $AcO-I$ assays

Methods described by Nadeau & Eicher (1982) were used for typing $GalT$ and $AcO-I$.

3. Results and discussion

To measure the recombination frequency between $GalT$, $AcO-I$, and brown ($b$) in a linkage cross not involving a Robertson translocation, C57BL/6J ($GalT^a$ $AcO-I^a$ $b^+$/ females were crossed to ST/bJ
Allelic combination

| Allelic combination | No. observed |
|---------------------|--------------|
| Galt b Aco-l a      | 65           |
| Galt a Aco-l b      | 93           |
| Galt b Aco-l c      | 2            |
| Galt a Aco-l c b    | 2            |
| Galt a b Aco-l B    | 19           |
| Galt b a Aco-l B    | 18           |

Recombination frequency

| Galt–Aco-l:      | 4/199 = 0.020 ± 0.010 |
| Aco-l–b:         | 37/199 = 0.186 ± 0.028 |
| Galt–b:          | 41/199 = 0.206 ± 0.029 |

(Galt\(^{a}\) Aco-1\(^{b}\) b) males and F\(_1\) hybrid males were backcrossed to DBA/2J (Galt\(^{a}\) Aco-1\(^{b}\) b) females. Progeny were typed for Galt, Aco-1, and brown (b) whose location on Chr 4 is well established (Cattanach, 1978; Nadeau & Eicher, 1982; Nadeau et al. 1986). A total of 199 progeny were typed (Table 1). GALT and ACO1 were reyped in each putative recombinant to verify the initial typing. Double crossovers were not observed. The most likely gene order and recombination frequencies were Galt–0.020 ± 0.010–Aco-1–0.186 ± 0.029–b.

Recombination data can be used to estimate the length of the chromosomal segments that have been conserved since divergence of lineages leading to mouse and man. It is usually assumed that these segments are not interrupted by unrelated genes. Genes on Chromosome 9 in man may represent an exception. Although many of the murine homologues of genes on human Chromosome 9 are located on mouse Chromosome 4, the homologues of several others, e.g. Abl, Ak-1 and Fpgs, are located distal to GALT and ORM1. In the mouse, the weighted average recombination frequency between Galt and Aco-1 is 0.043 ± 0.001 (9/305), combining data from Nadeau et al. (1986) and this study, and between Aco-1 and Lv 0.12 (Nadeau et al. 1986). Therefore the recombination frequency between Galt and Lv, the outermost genes marking the conserved segment, is 0.15. By using equation 2 of Nadeau & Taylor (1984), the length of the conserved chromosomal segment marked by three genes is estimated to be 24 cM.

Ovarian teratoma mapping provides a reliable way to estimate centromere–gene recombination frequencies (Eicher, 1978; Eppig & Eicher, 1983; Ott et al. 1976). We used this method to estimate the centromere–Galt recombination frequency and, by comparison with recombination frequencies in previous crosses involving a Robertsonian translocation, determine whether Rb2 suppresses recombination on the proximal portion of mouse Chr 4. These studies required construction of an LT/Sv congenic strain with a variant allele of Galt. To construct this strain (LT/Sv [Galt\(^{a}\) × ST/bJ [Galt\(^{b}\)])F\(_1\) hybrid females were backcrossed to LT/Sv males. Blood samples were collected from backcross progeny and typed for GALT. Male or female Galt\(^{c}\)/Galt\(^{b}\) heterozygotes were selected and backcrossed to LT/Sv. This process was repeated for 4 backcross generations. Both male and female Galt\(^{c}\)/Galt\(^{b}\) heterozygotes were then crossed to the recombinant inbred strain LT/Sv (Galt\(^{a}\)). Of these F\(_1\) hybrids, 80–90 % have spontaneous ovarian teratomas (J. H. Nadeau and D. S. Varnum, unpublished observations). Virgin F\(_1\) hybrid females that were heterozygous for Galt were autopsied when they were 70–90 days old. Teratomas were removed and typed for Galt. A kidney from each female in which a teratoma was found was also typed to verify maternal heterozygosity for Galt. The frequency of heterozygous teratomas was 0.48 (Table 2). Ott et al. (1976) showed that the recombination frequency between the centromere and a gene is immeasurable if the frequency of heterozygous teratomas is greater than 0.33. In addition, they showed that the frequency of heterozygous teratomas approaches 2/3 as the number of crossovers between the centromere and the gene increases. Because the observed frequency of heterozygous teratomas (0.48) exceeds 0.33, the recombination frequency between the centromere and Galt

| GALT phenotype | No. observed |
|----------------|--------------|
| GALT-AB        | 15           |
| GALT-A         | 8            |
| GALT-B         | 8            |
is immeasurable. The distance between the centromere and Galt is probably large, however, because the frequency of heterozygous teratomas exceeds 0.33.

A comparison of the recombination frequency between the centromere of Chr 4 and Galt in standard linkage testing crosses in which Rb2 segregated and in ovarian teratoma mapping experiments that did not involve a Robertsonian translocation demonstrated that Rb2 suppressed recombination on the proximal portion of mouse Chr 4. The recombination frequency between the centromere and Galt was 0.033 ± 0.020 in a linkage cross involving Rb2 (Nadeau & Eicher, 1982), whereas Galt appeared to be unlinked to the centromere in the present ovarian teratoma mapping experiment (Table 2). This difference in recombination frequency for the interval proximal to Galt was highly significant ($\chi^2 = 23.00$; $P < 0.005$), assuming that the high frequency of heterozygous teratomas corresponds to a recombination frequency of at least 0.165 (c.f. Ott et al. 1976). The $\chi^2$ value is an underestimate because the recombination frequency between the centromere and Galt was probably greater than 0.165. By contrast, recombination distal to Galt was not suppressed. The recombination frequency between Galt and Aco-1 was 0.047 ± 0.021 in a linkage cross involving Rb2 (Nadeau & Eicher, 1982), where the frequency was 0.020 ± 0.010 in the present linkage testing cross that did not involve a Robertsonian translocation (Table 1). This difference in recombination frequencies for the interval distal to Galt was not significant ($\chi^2 = 2.06$; $P > 0.05$). Rb2 therefore suppressed recombination proximal but not distal to Galt.

Previously, Cattanach (1978) argued that Rb2 suppressed recombination between the centromere and b. Location of the gene audiogenic seizure prone asp) near the centromere of mouse Chr 4 (Collins, 1970) was essential to Cattanach's argument for suppression. The gene asp is now believed to be located on Chr 12 (Seyfried & Glaser, 1981; Lusis et al. 1987). Therefore, Cattanach's results do not provide evidence that Rb2 suppresses recombination. Results of the present study, however, clearly demonstrate that Rb2 suppresses recombination between the centromere and Galt.

Genomic DNAs have been preserved from most of the animals in the linkage cross. Individuals interested in using these DNAs for mapping molecular markers should contact J.H.N.

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