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# Regional Localization for *HLA* by Recombination with a Fragile Site at 6p23

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SUMMARY

A family with a fragile site on chromosome 6 at band p23 was examined for recombination between the fragile site and *HLA*. Recombination was observed in four of the 20 offspring in whom it could occur. The estimate of the genetic length of chromosome between the fragile site and *HLA* is 20 centimorgans (cM) with a lower 95% probability limit of 8.5 cM, placing *HLA* proximal to the midpoint of 6p22. The most likely regional localization is at 6p21.3, which agrees closely with methods that do not involve recombination with the fragile site. This fragile site does not measurably disrupt recombination frequency, and the allele predisposing to expression of the fragile site is situated at the fragile site.

## INTRODUCTION

A family segregating for a fragile site in the distal region of band p23 on chromosome 6 provided an opportunity to localize *HLA* by measuring the recombination rate between *HLA* and the fragile site. The regional localization for *HLA* on chromosome 6 between p21.2 and p23 has been well established [1], and there is strong evidence for its elimination from the distal portion of this interval [2–6]. Given a proximal limit for *HLA* at 6p21.2, the distance between *HLA* and the fragile site must be less than 25 map units according to the male meiotic map presented by Cook et al. [7]. Hence, linkage between *HLA* and this fragile site can be assumed.

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## MATERIALS AND METHODS

Part of this family and the fragile site at 6p23 have been previously described using standard procedures for detection of folate-sensitive fragile sites [8]. At least 50 metaphases were routinely scored for the presence of fragile sites. *HLA* haplotypes at the A, B, and C loci were determined by the microcytotoxicity test. Chromosome breakpoints defined using different nomenclature systems have been standardized to the ISCN system [9]. It is assumed that the relationship between map distance and recombination frequency is approximately linear up to $\theta = .25$, and that recombination is often greater in females than in males [10].

## RESULTS

Four generations were available for study (fig. 1). The fragile site at 6p23 was detected without difficulty in every generation. Eight carriers were detected, and one untested individual (II.11) was an obligate carrier. It is highly improbable that the other untested, unrelated individuals (I.3, II.4, II.12) would carry the fragile site since this is the only family in which this fragile site has been recorded. The frequency of expression was 44% for the propositus (II.13), and ranged from 7% to 30% of cells for the other carriers. For the purpose of linkage analysis, the fragile site in this family may be regarded as a heritable codominant variant.

The inheritance of the fragile site and *HLA* haplotypes are shown in figure 1. *HLA* haplotypes have been coded as indicated in table 1. The HLA phenotypes could be inferred in the untested individuals as 7/8 in I.3, 6/? in II.4, 2/8 in II.11, and 11/12 in II.12. There were three nonrecombinants (NR) between *HLA* and the fragile site in generation IV. There were seven NR and two recombinants (R) in generation III. Given prior knowledge of linkage, it is highly probable that there are six NR and two R in generation II rather than six R and two NR.

The combined male and female recombination frequency ($\theta$) is 20% (4/20), well within the maximum expected male recombination frequency of less than

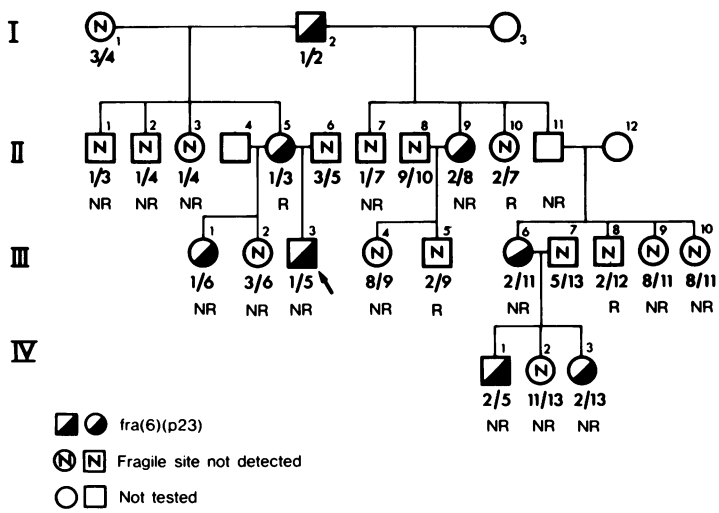


FIG. 1.—Inheritance of *HLA* and the fragile site at 6p23 with recombinants (*R*) and nonrecombinants (*NR*) indicated. See text for probable phenotypes of untested individuals.

TABLE 1

*HLA* HAPLOTYPE CODE

| Code | Haplotype |
|---|---|
| 1..... | *Aw31, Bw57, Bw4, Cw6* |
| 2..... | *A1, Bw49, Bw4* |
| 3..... | *A1, B8, Bw6* |
| 4..... | *Aw32, B27, Bw4, Cw1* |
| 5..... | *A2, Bw44, Bw4, Cw5* |
| 6..... | *A11, Bw22, Bw6, Cw3* |
| 7..... | *A29, B7, Cw6* |
| 8..... | *A3, B7, Bw6* |
| 9..... | *A26, Bw41, Bw6* |
| 10..... | *Aw33, B14* |
| 11..... | *Aw30, B18, Bw6, Cw5* |
| 12..... | *A2, Bw35, Bw6, Cw4* |
| 13..... | *A28, B27* |

25%. The maximum lod score was 1.7 at $\theta = 0.2$. The recombination frequency is equivalent to map distance expressed as cM because of the approximate linear relationship between map distance and $\theta$ up to $\theta = .25$. The lower 95% probability limit for this interval is 8.5 cM and was determined by subtracting 5% of the area under the truncated relative probability curve from one end of the curve. The curve was truncated at 6p21.2. Given that recombination frequency is often greater in females than in males, the estimate of 20 cM for the interval between *HLA* and the fragile site at 6p23 is possibly an overestimate for comparison with a map expressed in male cM. However, in this family, only one out of eight offspring of informative females were recombinants, compared to three out of 12 from informative males. These results suggest a probable regional localization for *HLA* between about the midpoint of 6p22 (near to 6p22.2) and the previously known proximal limit for *HLA* at 6p21.2. *HLA* is not tightly linked to the fragile site and is unlikely to lie within 6p23 or the distal half of 6p22. The most likely location of *HLA* is at 6p21.3 (fig. 2).

## DISCUSSION

Considerable information already exists for the regional localization of *HLA*. Family study with a translocation t(6;21)(p22;q11) suggested the probable localization of *HLA* proximal to 6p22 [2]. A translocation family with t(6;20)(p21;p13) demonstrated close linkage ($\theta = .05$) between the breakpoint (in 6p21 near 6p22) and *HLA* [3]. While the effect of reciprocal translocations on recombination is not definitely known, there is unlikely to be any major disruption [11]. The absence of a duplicated set of parental HLA antigens in a child partially trisomic for 6p22.2→pter derived from a balanced translocation t(6;10)(p22.2;pter) excluded *HLA* from 6p22.2→pter [5]. Subsequently, *HLA* was localized to 6p21 [6]. Berger et al. ([4] and personal communication, 1982) proposed a more precise localization for *HLA*, suggesting that a chromosomal break at 6p21.2 or at the interface of 6p21.2 and 6p21.3 was within the *HLA* cluster. These data agree closely with the most likely position for *HLA* determined by recombination with the fragile site.

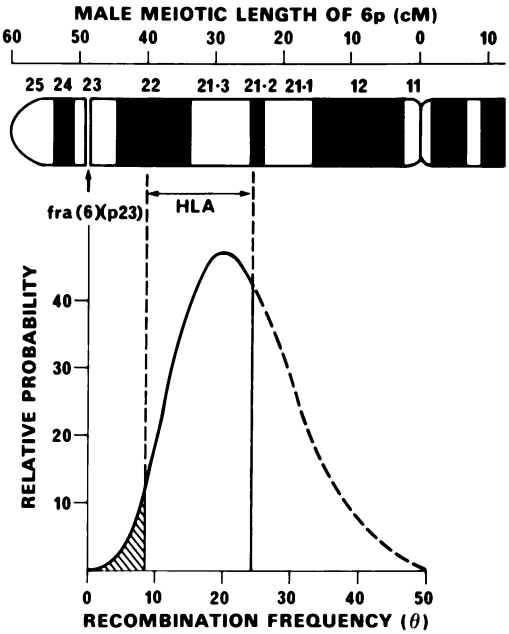


FIG. 2.—Probable localization of *HLA* by recombination with the fragile site at 6p23

The intervals between the fragile sites at 10q23 and 10q25 in families with both fragile sites [12], and now between *HLA* and the fragile site at 6p23, have been estimated by recombination and fall within limits established by other procedures. Consequently, fragile sites do not appear to disrupt recombination in chromosomal segments near the loci of expression and thus are valid markers for linkage analysis giving unbiased recombination values. Hence, the distance between *HP* and the fragile site at 16q22 as estimated by recombination between them [13] is probably a true indication of their distance apart.

The alleles responsible for the expression of fragile sites are probably situated on the chromosomes at or very near to the fragile site. This had been verified for the fragile sites at 10q23, 10q25 [12], and now at 6p23. The interval between genes responsible for the expression of fragile sites at 10p23 and 10q25 was estimated by recombination and is consistent with that expected from their locations on mitotic chromosomes. The positive lod score between *HLA* and the fragile site at 6p23 is consistent with existing knowledge of the location of *HLA* and the position of the fragile site at 6p23. Control of fragile site expression must be within the homolog expressing the fragile site since confirmed heterozygous carriers do not express fragile sites in both homologs.

The rapid rate of discovery of fragile sites [8] indicates that many more of these are likely to exist and be used for linkage studies. Fragile sites have advantages over other chromosomal variants because they are not restricted to paracentromeric regions. They are precisely mapped by banding and are ideal both for use in searching for linkages with unassigned markers in chromosome regions devoid of polymorphic genetic markers and for the regional localization of markers

shown to be near regions of fragile site expression. The fragile site at 6p23 confirms a more exact regional localization for *HLA* and, consequently, the cluster of genes that are known to be linked to *HLA*. Confirmation that the location of DNA responsible for a fragile site is at the locus of expression may now permit the characterization of this DNA by study of the relevant chromosomal segments.

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