Chiasma derived genetic maps and recombination fractions: chromosome 13 with reference to the proposed 13q14 retinoblastoma locus

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SUMMARY Provided that there is no chromatid interference, no movement of chiasmata, and no discrepancies between meiotic and mitotic chromosome lengths, then genetic maps and recombination fractions may be directly derived from our meiotic chiasma distribution data. This is illustrated by male chiasma derived genetic lengths and recombination fractions along chromosome 13. The recombination fraction between 13p fluorescent markers and the proposed retinoblastoma locus at 13q14 is estimated at 0.27 to 0.37 and preliminary female chiasma studies suggest a recombination fraction of 0.5 between these two sites. Therefore, it seems unlikely that 13p fluorescent markers may be of any practical help in identifying retinoblastoma gene carriers. This is also borne out by the discordant segregation which has been found in six out of seven retinoblastoma families, which gives a calculated recombination fraction of 0.39 (SE 0.15), not significantly different from 0.5.

All bilateral and 10 to 12% of unilateral cases of retinoblastoma are believed to be preconditioned by a germ line mutation which may be inherited as an autosomal dominant trait.¹ It has been proposed that the gene for retinoblastoma (Rb-1) is located on the long arm of chromosome 13, because some patients have a constitutional deletion of 13q involving band 14.² By analysis of banded prophase chromosomes the deletion was pinpointed to the central part of band 13q14 in one case of unilateral retinoblastoma.²

Recently, Knight *et al*³ have traced the inheritance of chromosome 13 in a number of families segregating for retinoblastoma using fluorescent 13p markers. It was hoped that it would be possible to observe concordant segregation of the gene Rb-1 with one of the chromosomes 13 of the affected parent. Three of the four families, however, showed discordant segregation, which implies that either the gene for this disorder is not on chromosome 13, or that there is a significant amount of crossing over between the Rb-1 locus and the fluorescent marker. Similar data of discordant segregation in all their three families are reported by Morten *et al*.⁴

If the physical location of a gene is known, then it is possible to estimate the amount of recombination between the gene and any other physical chromosomal landmark by counting chiasmata at

meiosis (see review⁵ as regards the situation in the human). In this paper we have illustrated this by estimating the expected recombination frequency between the 13p fluorescent markers and band 13q14, using chiasma distribution data from a human male with normal meiosis.⁶ The expected number of recombinants was found to be high and we are, therefore, not surprised by the high frequency of discordant segregation observed by Knight *et al*³ and Morten *et al*.⁴ Their findings are not contradictory to a 13q14 location for Rb-1.

Materials and methods

MEIOTIC ANALYSES

In preparations of human male meiosis it is possible to identify individual bivalents⁷ and measure the positions of the chiasmata, for example, relative to the centromere.⁶ Details of meiotic chromosome preparation methods and also the methods of chiasma scoring have been described previously.^{5,6} The data used in this study are from the person described in the paper by Hultén.⁶ The band positions have been taken from the measurements of the mitotic chromosome by Francke and Oliver.⁸

ESTIMATION OF RECOMBINATION FRACTIONS AND GENETIC MAP DISTANCES

Each chiasma between two loci on a chromosome

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represents a single recombination event and as such is expected to produce two recombinant and two non-recombinant gametes. If there is no chromatid interference then two or more chiasmata between the two loci will still on average produce 50% recombinant and 50% non-recombinant gametes. The expected proportion of recombinant offspring, the recombination fraction, may then be calculated as half the proportion of cells with one or more chiasmata between the two loci. Mather⁹ has expressed this relationship as the formula $p = \frac{1}{2}(1-a)$, where p is the recombination fraction and a is the proportion of bivalents having no chiasmata between the two loci concerned. The recombination fraction, therefore, tends to $\frac{1}{2}$ as a approaches zero and to 0 as a approaches 1.

The genetic distance between two loci on a chromosome is normally expressed in centiMorgans. A Morgan is by definition that length of a chromatid which has experienced, on average, one crossover event per meiotic division.¹⁰ Since each chiasma only affects two of the four chromatids, genetic distance in Morgans can be estimated by halving the mean chiasma frequency between the two loci.

FIG 1 Chiasma frequency distribution along 13q based on data from 35 cells. Each point represents the chiasma frequency within the band. The chiasma positions were measured on the unbanded meiotic chromosome⁵ and then assigned to the bands⁸ according to their relative distances.

Results

CHIASMA ANALYSIS OF CHROMOSOME 13

Three of the 35 cells analysed had a chiasma in the short arm which in each case was distally located. It is difficult to tell whether these chiasmata would be proximal to the 13p fluorescent markers used in the family studies because the latter are composite and involve the whole short arm. However, all three cells also had a proximal chiasma in 13q, in which case the short arm chiasma would not alter the recombination values (see Materials and methods). For simplicity, therefore, the 13q arm only is illustrated.

Fig 1 shows the frequency distribution of chiasmata along the long arm of chromosome 13 based on data from 35 cells. The chiasmata are unevenly distributed along the chromosome arm with proximal and distal peaks. The absence of chiasmata in the band just proximal to the end of the chromosome may be an artefact produced by the technical difficulties of measuring the precise positions of very distal chiasmata.

These chiasmata data were used to calculate recombination fractions (fig 2) and genetic distances (fig 3) between the centromere and points along 13q. It can be seen (fig 3) that there is a marked increase in genetic length relative to physical length in the distal part of 13q, corresponding to the high distal chiasma frequency.

PREDICTED RECOMBINATION FRACTIONS BETWEEN 13 cen AND POINTS ALONG 13q

The predicted male recombination fractions are given in the table and illustrated in fig 2. There is a cumulative increase from the centromere up to about 75% of the chromosome arm length, when a situation is approached where there is at least one chiasma per 13q in all cells scored. The recombination fraction will then amount to 50% and loci situated distally to this point are not expected to show co-segregation with centromeric or pericentromeric markers. Accordingly, it will not be possible to demonstrate linkage by family analysis for these markers.

PREDICTED RECOMBINATION BETWEEN 13p FLUORESCENT MARKERS AND Rb-1

The Rb-1 gene is proposed to be located at band q14 of chromosome 13. This band ranges from 25% to 42% of the long arm away from the centromere.⁸ The chiasma distribution on chromosome 13 was analysed in a sample of 35 cells and there were 19 and 26 chiasmata, respectively, between the centromere and these two reference points. No bivalents had more than one chiasma in this region, while, as already mentioned, three bivalents had an additional

FIG 2 Expected recombination fractions between the centromere and points along chromosome 13q estimated from the chiasma data. Each point represents the value up to the border of a chromosome band.

FIG 3 Genetic distance between the centromere and points along chromosome 13q estimated from the chiasma data. Each point represents the genetic distance up to the border of a chromosome band.

chiasma on 13p. The range of the recombination frequencies between the centromere and Rb-1 is calculated as $\frac{19/35}{2}$ to $\frac{26/35}{2}$, that is 0.27 to 0.37 (table), and the same values are reached when the

TABLE Chiasma derived recombination values between 13 cen and band borders on 13q

Region	Band	Relative distance*	Cumulative chiasma frequency	Recombination fraction	Genetic distance (cM)
1	Centromere	0	0	0	0
	1	0.02	0	0	0
	2	0.16	0.37	0.185	18.5
	3	0.25	0.54	0.27	27.0
	4	0.42	0.74	0.37	37.0
2	1	0.60	0.91	0.43	45.5
	2	0.67	1.00	0.485	50.0
3	1	0.78	1.06	0.50	53.0
	2	0.88	1.14	0.50	57.0
	3	0.93	1.14	0.50	57.0
	4	1.00	1.77	0.50	88.5
	Telomere				

* From Francke and Oliver.⁸

chiasmata on 13p are included. In other words, between 27% and 37% of the children of an affected person would be expected to show recombination between a 13p fluorescent marker and the Rb-1 gene.

Assuming that the Rb-1 locus designation at 13q14 is correct, the pedigree data given by Knight *et al*³ and Morten *et al*⁴ may be used to calculate the recombination fraction between the gene Rb-1 and a fluorescent marker on 13p. If we take families 1 and 2 of Morten *et al*,⁴ together with families 1 to 4 of Knight *et al*,³ assuming that subject II.2 in family 4 is a non-manifesting heterozygote, we find, in the usual way¹¹ that the likelihood of obtaining the observed results is proportional to

$$I = 18 (1-\theta)^8 \theta^7 [\theta^2 (1-\theta)^2] [(1-\theta)^4 + \theta^4]$$

where θ is the recombination fraction. Our best estimate of the recombination fraction is the value of θ which maximises I : this value is 0.386. The standard error of this estimate is 0.154, so that the estimated recombination fraction does not differ significantly from 0.5.

Discussion

It is a paradoxical task to try to tie up any particular gene locus on a chromosome by co-segregation of the genetic condition and a chromosomal marker in case they may be so located that there is a lot of recombination between them. The proposed recombination fraction of between 0.27 and 0.37 obtained from male chiasma data is to be compared with the corresponding empirical family data, which give a

recombination value of 0.39 (SE 0.15) which is not significantly different from 0.5.

Accordingly more extensive family material would be needed to demonstrate true linkage between the 13p markers and Rb-1, and if the locus is in fact located more distally on 13q we approach a situation where male chiasma data predict recombination fractions approaching 50%, in which case no linkage is expected.

Cook *et al*¹¹ have similarly found only weak linkage between 13p fluorescent markers and esterase D, also assigned to 13q14. Their findings give a male recombination frequency of 0.44 based on eight informative offspring, while the female recombination frequency is 0.47 calculated from 27.

We may conclude that the family data and chiasma data are in general agreement, and any discrepancies seem more likely to be the result of the small amount of data than of any basic methodological faults.

How reliable then are the chiasma data? Previously, it has been thought that chiasmata might already have moved from their original positions at the time when they are scored,⁹ and thus they would no longer represent recombination sites, but this idea has recently been refuted in a number of organisms ranging from grasshoppers to mice^{12 13} and there are indications that man is unlikely to be an exception in this respect.^{5 6 14}

The accuracy with which chiasmata may be scored has recently been discussed by Hultén *et al*.⁵ It is of primary importance to note that the chiasma positions have not been directly assigned to chromosome bands on the meiotic chromosome, but measured and related to the mitotic chromosome bands via the relative distances as measured by Francke and Oliver.⁸ One of the problems is, therefore, the possible lack of correspondence in physical length between the mitotic and meiotic chromosome. Perhaps we should add that in the initial investigation we found a high correlation ($r=0.99$) between total relative lengths of the respective mitotic and meiotic chromosomes, but the agreement of centromere indices was numerically lower ($r=0.95$).

The calculations presented in this communication were made using chiasma data without any mathematical modification¹⁵⁻¹⁷ from a single person. Until recently, we were concerned that there might be significant differences in chiasma distribution patterns between subjects, with the effect that it would be difficult to use the limited chiasma data available to construct general genetic maps, and impossible to predict recombination fractions from one subject to another until a much larger chiasma material had been obtained. We were therefore somewhat relieved when we found that there are no dramatic differences in chiasma locations in a

sample of seven men, but this investigation was limited to chromosomes 1, 2, and 9.¹⁴ Our general impression from a larger number of subjects is that the conformity would also include chromosome 13, but this warrants further analysis, particularly with respect to the possible influence of heteromorphic variants involving heterochromatin.

A perhaps more serious limitation to the chiasma data is that they are largely restricted to human males. Preliminary information from human females indicates that the long acrocentrics including chromosome 13 might have a higher number of chiasmata than in the male.⁵ Our impression is that most oocytes would have either two or three chiasmata in the long arm of chromosome 13 of which one always would be located proximal to 13q14. In females, the expected recombination fraction may approach 50% and no linkage between pericentromeric markers and the proposed retinoblastoma locus at 13q14 would be expected.

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