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MULTIPOINT MAPPING STUDIES OF THE β-GLOBIN, INSULIN, AND C-HA-RAS-1 LOCI ON 11P

To the Editor: We wish to comment on our previous linkage studies on the three loci: β-globin (HBBC), insulin (INS), and oncogene c-Ha-ras-1 (HRAS1) assigned to 11p [1], and present some further multipoint analyses of these loci. The HBBC loci are approximately 10 cM from the pair of loci, HRAS1 and INS. The order of the last two loci, which are 2–4 cM apart, has been a matter of controversy [1–4].

The multipoint relationships of these loci has now been examined in detail using the new computer program LINKAGE, version 3.1 [5, 6] and published data [1]. Two approaches to multipoint mapping were utilized. The first gave the maximum likelihood estimate (MLE) of the map distance of a locus in relationship to two loci whose linkage relationship was assumed (subprogram LINKMAP). Map distances were used instead of recombination fractions. No interference of crossing over was assumed; Haldane's mapping function was used [7]. The order of the loci was analyzed by setting the distance between the HBBC and HRAS1 loci (based on previous two-point results at 9 cM [1]) and determining the MLEs of the map distance of INS from these two fixed loci. As seen in figure 1, the MLE of the map distance placed the INS locus 8 cM from the HRAS1 locus and 16 cM from HBBC, giving the order HBBC:HRAS1:INS. However, only a small difference in the $\log_{10}$-likelihood (0.96) was seen when HRAS1 was distal to INS and β-globin vs. when HRAS1 was located between β-globin and INS (fig. 1). Similar results were obtained when the distance between the HBBC and INS loci was fixed at 12 cM, and the MLE of the map distance of HRAS1 relative to these loci was determined. The most likely order was HBBC:HRAS1:INS with a map distance of 5 cM between HRAS1 and INS. In this analysis, a very small difference in the $\log_{10}$-likelihoods was found ($\approx$ 0.2) between this order and the order HBBC:INS:HRAS1. It should be remembered that in using this approach the distance between two of the three loci is fixed; that is, the data are analyzed only for the relationship of a third locus with respect to the two fixed loci, not for the linkage relationship between

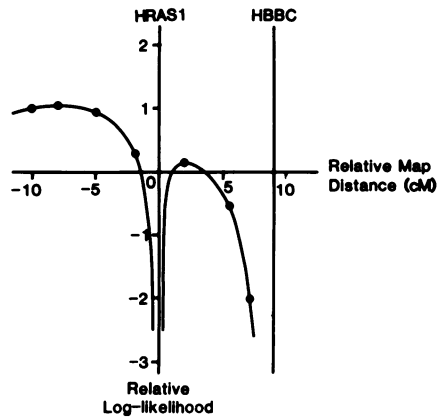

FIG. 1.—Relationship of the INS locus to HRAS1 and HBBC. The relative $\log_{10}$-likelihoods are shown for different map positions of the INS locus in a relationship to the map of the HRAS1 and HBBC loci.

the two loci at a fixed distance. The differences between the likelihoods for different orders of the three loci will give the most likely order of the loci under this constraint. However, the $\log_{10}$-likelihoods obtained from these orders may be quite different from those obtained by joint analysis of the data from all three loci.

The second multipoint approach was to look at the joint likelihoods for the three loci, HBBC, HRAS1, and INS, using the subprogram MLINK of LINKAGE. This was done first under the assumption of no interference and then by using the Kosambi mapping function that postulates a relatively low level of interference [7], assuming equal recombination in males and females. The results are presented in table 1. The $\log_{10}$-likelihoods are slightly larger for the order HBBC:HRAS1:INS under the assumption of no interference (1.033 vs. 0.642) or with the Kosambi mapping function (0.909 vs. 0.526). However, these differences in $\log_{10}$-likelihoods are certainly not large enough to definitively order these loci.

The two-point results suggested the order INS:HRAS1:HBBC as did previ-

TABLE 1

MULTIPOINT ANALYSES OF THE HBBC, HRAS1, AND INS LOCI ON 11p

ORDER	RECOMBINATION FRACTIONS ($\theta_m = \theta_f$)			RELATIVE LOG L*
	HBBC: INS	HBBC: HRAS1	INS: HRAS1	
A. No interference:				
HBBC:HRAS1:INS	0.16	0.08	0.08	1.033
HBBC:INS:HRAS1	0.10	0.15	0.05	0.642
B. Kosambi level of interference:				
HBBC:HRAS1:INS	0.16	0.08	0.08	0.909
HBBC:INS:HRAS1	0.10	0.14	0.04	0.526

*Relative $\log_{10}$-likelihood.

ous three-point analyses where a relatively high level of interference was assumed [1]. In these new multipoint analyses, slightly better $\log_{10}$-likelihoods were still obtained for the previously postulated order INS:HRAS1:HBBC. However, analyses of the Utah families showed evidence for the order HBBC:INS:HRAS1 [2]. The Utah database is more extensive than ours and the families were typed at an additional locus ADJ, which maps closer to INS and HRAS1 than HBBC does. It is easier to determine the order of two tightly linked loci such as INS and HRAS1 when there is a third locus in the same region, since double recombinant offspring become less likely. We now believe that our previous analyses placed too much emphasis on phase unknown, multiply heterozygous families in an attempt to order the INS and HRAS1 loci with respect to the HBBC locus [1]. We were incorrect in our interpretation of one our families (no. 18 in [1]), which, in fact, does not give any information on gene order. We wish to thank Dr. K. Kidd and his associates at Yale University for bringing this to our attention. As demonstrated by Lathrop et al. [4], odds obtained in favor of any given order can vary greatly depending on the level of interference of crossing over used in the analyses. In summary, all of our analyses give evidence for the order HBBC:HRAS1:INS; however, the evidence for this order is not significant. Analyses of the more extensive Utah data provide more convincing evidence for the order HBBC:INS:HRAS1.

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