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Brief Communication

Linkage of DNA Probe B79a (D7S13) to Cystic Fibrosis

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SUMMARY

We have conducted, in 64 affected families, a study of linkage between the anonymous DNA segment pB79a (D7S13) and the locus for cystic fibrosis (CF) on chromosome 7q. The maximum lod score was 12.60 at $\theta = .08$ (confidence bounds .045–.135). Although D7S13 is not sufficiently close to CF for routine use in DNA-based prenatal diagnosis, it will be helpful in certain families when other nearby markers are uninformative. D7S13 will also be useful for refining the linkage map of the CF region.

INTRODUCTION

Cystic fibrosis (CF) is a common autosomal recessive disease in Caucasian populations. Genetic linkage has been demonstrated previously between six polymorphic DNA sequences and the CF gene (*CF*), (Scambler et al. 1985, 1986; Tsui et al. 1985; Wainwright et al. 1985; White et al. 1985). Linkage mapping has placed three of these sequences—pJ3.11 (D7S8), p*met*H (MET), and p7C22 (D7S16)—≤4 centimorgans from *CF* (Beaudet et al. 1986; Scambler et al. 1986), and their physical localization (Dean et al. 1985; Bartels et al. 1986; Buckle et al. 1987) suggests that the *CF* gene maps to chromosome 7q22-

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7q31.1. A recent report from several centers, consisting of pooled linkage data for *CF*, D7S8, and MET, has suggested that these two DNA markers probably blank the *CF* locus (Beaudet et al. 1986).

Here we report genetic linkage between *CF* and the polymorphic DNA sequence pB79a (D7S13) (Bartels et al. 1986; Estivill et al. 1986) in affected families from Canada, the United Kingdom, and the United States. Analysis of haplotypes shows random association (no linkage disequilibrium) between alleles at the D7S13 and the *CF* loci. However, the linkage of D7S13 to *CF*, although not close enough for routine diagnosis, provides an additional marker to define the region. Linkage analyses involving multiple loci including D7S13 will be an important component of efforts to identify the *CF* gene.

MATERIAL AND METHODS

Description of Probe pB79a (D7S13)

pB79a is a 1.2-kb *Hin*dIII-*Eco*RI fragment cloned into pBR328 (Bartels et al. 1986). It has been mapped to 7cen-q22 by Bartels et al. (1986) by means of a series of cell hybrids and has been shown to be missing in a patient hemizygous for a deletion of 7q22-q32 (Estivill et al. 1986). This probe detects *Hin*dIII and *Msp*I polymorphisms.

Family Studies

Sixty-four families, each with at least two CF-affected sibs, were informative in the linkage study with D7S13. DNA samples were digested with the appropriate restriction enzyme, fractionated on agarose gels, blotted, and hybridized according to a method described elsewhere (Estivill et al. 1986). Linkage analysis was performed with the LINKAGE program (Lathrop et al. 1984). Haplotypes for the *Hin*dIII and *Msp*I polymorphisms were used where appropriate in the analysis. Haplotypes at the D7S13 and *CF* loci were counted in families in whom both parents were typed and phase was unambiguous, to test for linkage disequilibrium.

Polymorphic information content (PIC) for the D7S13 haplotype was calculated according to the formula proposed by Botstein et al. (1980). The significance of disequilibrium between the polymorphisms was calculated with Fisher's exact test, and the standardized disequilibrium coefficient was calculated according to the method of Hill (1975).

RESULTS

The lod scores at selected recombination levels for D7S13 and CF are listed in table 1. A maximum-likelihood lod score of 12.60 was calculated, with a recombination fraction in both males and females of .08. Confidence lower and upper bounds for recombination between *CF* and D7S13 polymorphisms, calculated according to the Human Gene Mapping 8 convention, were .045 and .135.

Highly significant disequilibrium was indicated between the *Hin*dIII and

TABLE 1

SELECTED LOD SCORES AT SEVERAL RECOMBINATION FRACTIONS, θ_m, $\theta_f = \theta_m$

SOURCE (N) OF INFORMATIVE FAMILIES	LOD SCORE AT RECOMBINATION FRACTION					
	.01	.05	.10	.20	.30	.40
Salt Lake City (22)	3.96	5.78	5.72	5.12	2.44	0.75
London (18)	5.56	5.96	5.30	3.48	1.76	0.49
Toronto (24)	−4.55	0.09	1.33	1.49	0.88	0.26
Total (64)	4.97	11.83	12.35	10.09	5.08	1.50

TABLE 2

HAPLOTYPE FREQUENCIES FOR *D7S13* AND *CF*

ALLELE PAIRS[a] (*Hin*dIII/*Msp*I)	NO. OF CHROMOSOMES (CF/Normal)			
	Salt Lake City	London	Toronto	Combined
1/1	4/3	1/1	6/6	11/10
1/2	0/0	0/2	1/0	1/2
2/1	4/10	9/7	13/10	26/27
2/2	30/25	14/14	37/43	81/82
Total	76	48	116	240

[a] *Hin*dIII allele 1 = 8.1 kb; *Hin*dIII allele 2 = 4.3 kb; *Msp*I allele 1 = 8.4 kb; and *Msp*I allele 2 = 11.6 kb.

*Msp*I polymorphisms at the D7S13 locus ($P < .00001$; standardized disequilibrium coefficient .41). The PIC for this locus is .48.

Haplotype frequencies for CF-D7S13(*Hin*dIII)-D7S13(*Msp*I), charted in table 2, show no evidence for disequilibrium between the D7S13 polymorphisms and *CF*.

DISCUSSION

The linkage data presented here show D7S13 to be ~8 centimorgans from the *CF* locus. Our data provide no significant evidence of linkage disequilibrium between D7S13 and *CF*, a result consistent with the estimated genetic distance between the loci. Although linkage is not sufficiently tight for routine DNA-based prenatal diagnosis, D7S13 will be helpful in certain families when other markers are uniformative.

The D7S13 locus will be useful for refining the linkage map in the vicinity of the *CF* gene. More precise localization of *CF* with respect to the closely linked DNA markers will increase the accuracy of the markers for prenatal diagnosis, and refinement of the genetic map of this region could also suggest strategies for the eventual isolation of the *CF* gene.

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