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Isolation of a further anonymous informative DNA sequence from chromosome seven closely linked to cystic fibrosis

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ABSTRACT

A library prepared from flow-sorted chromosomes was used to isolate single-copy sequences from chromosome seven. One such sequence 7C22 has been shown to be polymorphic for an EcoRI restriction site and to be informative for the study of CF in approximately 35% of matings. The segregation of the 7C22 alleles was followed through nineteen informative families with more than one child affected by cystic fibrosis. We report that the locus for 7C22 is linked to the locus for cystic fibrosis at a recombination fraction of 0.045. This marker will prove useful in improving the accuracy and informativeness of prenatal diagnosis and in constructing a fine genetic map around the cystic fibrosis gene.

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease characterised by chronic pulmonary disease, pancreatic insufficiency and elevated sweat electrolyes (1). Evidence suggests that the physiological defect involves a relative impermeability to chloride across sweat gland duct and respiratory epithelium (2,3) and a defective β-adrenergic response in affected tissues (4,5).

We have recently demonstrated linkage between the locus for the cystic fibrosis mutation (CF) and several markers on the long arm of chromosome seven. Tight linkage exists between the DNA sequence pJ3.11 and CF, and loose linkage with the collagen gene (COL1A2) and the gene encoding for the β chain of the T-cell receptor (TCRB) (6,7). Our Danish collaborators have shown linkage between CF and the locus of the serum enzyme paraoxonase (PON) (8). Two other groups have also reported linkage between CF and cloned DNA markers on chromosome 7: an anonymous DNA probe 917 (9) and the MET oncogene (10).

The two most closely linked markers, pJ3.11 and MET, should be useful for prenatal diagnosis and heterozygote detection of cystic fibrosis in families where there is a living sib affected by the disease. However, this approach is limited by the number and informativeness of the available

probes. In the case of CF, as for all autosomal recessive diseases, it is necessary to distinguish both homozygous and heterozygous unaffected from homozygous affected foetuses accurately. This is only possible when the informativeness of the available probes is sufficient to permit analysis of segregation of both mutant genes through the family. The development of a linkage map around CF using several probes could increase the accuracy of prenatal diagnosis as flanking markers may detect double recombination events.

In this paper we report the isolation of a further informative genomic DNA sequence from a chromosome seven-specific library which is closely linked to CF. This probe will be useful in the construction of a genetic map of the region flanking CF, and provide an additional starting point for attempts to isolate the gene defect itself.

MATERIALS AND METHODS

Derivation of 7C22

The chromosome seven-specific gene library used in this work was constructed at the Biomedical Sciences Division, Lawrence Livermore National laboratory, Livermore, CA 94550, under the auspices of the National Laboratory Gene Library Project, which is sponsored by the U.S. Department of Energy. The library obtained contains inserts cloned into the EcoRI site of phage Charon 21A. An aliquot of the original amplification of the library was used to prepare DNA by the method of lysis in liquid culture (11). A sub-library was constructed by subcloning into the vector SP64. Library DNA was digested to completion with EcoRI and fragments ligated into phosphatased vector by standard methods (12). After transformation and selection, ampicillin resistant colonies were picked and plated out in an ordered array for Grunstein and Hogness screening (13). Those colonies giving no detectable hybridisation with radioactively labelled total human DNA were picked for further analysis. Plasmid DNA was prepared from these colonies by alkaline lysis (14), digested with EcoRI and electrophoresed on 1% low-gelling temperature agarose gels. Inserts from recombinants were cut out of the gel and stored at -20°C.

Analysis of probes

Genomic DNA from ten unrelated individuals was prepared by the method of Kunkel et al. (15), digested to completion with various restriction enzymes and transferred bo Hybond^{N} membranes (Amersham International) using the method first described by Southern (16). Inserts were labelled by the method of

Feinberg and Vogelstein (17) to a specific activity of $1x10^9$ dpm ug^{-1}. Hybridisation, washing and autoradiography condition were as described previously (18).

Chromosomal localisation

A panel of 6 CHO x human hybrid cell lines were used for chromosomal localisation (18,19). Total human and CHO DNAs were run as controls.

Family Studies

31 families with at least two sibs affected by CF were used in this study. In total 73 affected and 32 unaffected children were available. DNA analysis was carried out as described above. Calculations were done using the LINKAGE program of Lathrop et al. (20).

RESULTS

The sub-library yielded around 1500 colonies. From these 200 were picked and analysed using the Grunstein and Hogness procedure; 65% of colonies gave no detectable hybridisation signal. Of the first 12 inserts studied eight were shown to be single copy sequences by hybridisation to genomic DNA.

Probe 7C22 has a 5.1 kb insert which demonstrates a two allele polymorphism with the enzyme EcoRI (Figure 1). The common allele (A1) is a 7.2 kb fragment and occurs with a frequency of 0.76 in a sample of 50 unrelated Caucasians. The rare allele is a 5.1 kb fragment of frequency 0.24. No polymorphisms were detected with the enzymes BamHI, NciI, ScaI, RsaI, TaqI, BclI, SacI, PvuII, BglII, PstI, MspI, HindIII, AvaII, XmnI, HaeIII, HincII, EcoRV and HinfI.

When hybridised to a panel of human X Chinese hamster ovary hybrid cell line DNAs probe 7C22 gave 100% concordance for chromosome seven only (Figure 2).

19 families were informative for linkage analysis between 7C22 and CF. Figure 3 shows the segregation of the 7C22 alleles in one family. The linkage data are presented in Table 1. The maximum lod score, assuming male

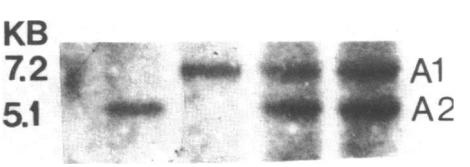

Figure 1 : Autoradiogram showing both homozygote forms, and the heterozygote form, of the EcoRI polymorphism.

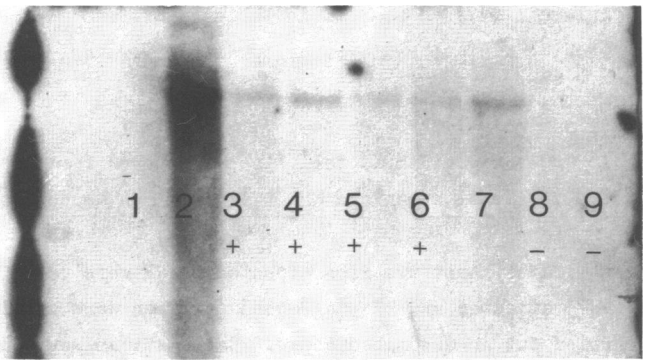

Figure 2 : Autoradiogram of 7C22 hybridised to hybrid cell line DNAs. Lane 1 - Chinese hamster ovary; 2 - 5 ug total human DNA; 3 = B4-2; 4 = GAL4; 5 = GAL7; 6 = Paw13; 7 = 2 ug total human DNA; 8 = DIS20; 9 = C4a12. +/- indicates presence of chromosome 7 in the hybrid in question.

and female recombination fractions (θ) to be equal, is 4.5 at 0.045. Using the LINKAGE program iteratively the maximum lod score is 4.55 at a male θ of 0.005 and a female θ of 0.07. This represents an odds ratio of 35550:1 in favour of linkage. The confidence limits ($p<0.05$) span θ = 0.0002 to θ = 0.22.

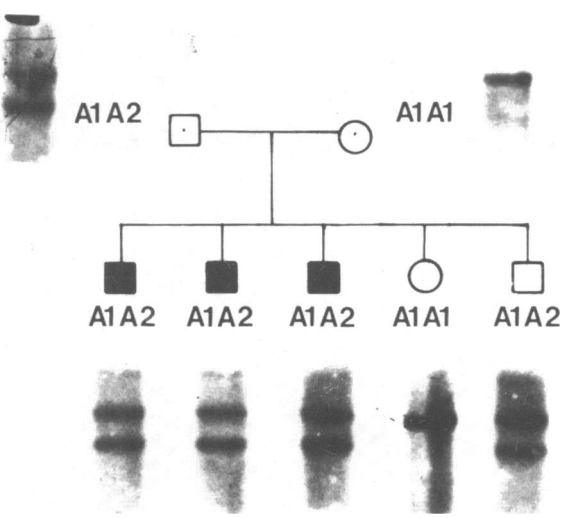

Figure 3 : Demonstrates the 7C22 alleles segregating in a pedigree with three affected children.

TABLE 1

θ	0.001	0.01	0.05	0.1	0.15	0.02	0.25	0.3
LOD	2.1	4.0	4.5	3.5	3.5	2.8	2.1	1.4

Lod scores for 7C22/CF linkage at various recombination fractions. Data for each family are available from the authors.

DISCUSSION

We have obtained a recombinant from a chromosome seven-specific library which shows linkage to CF. This brings the number of DNA probes linked to CF, at a recombination fraction of 0.05 or less, to three. The shortest region of overlap of these three probes is 7q21-q22 and it is likely that CF lies within these bounds. Altogether there are six DNA markers (917 (9), MET (10), pJ3.11 (6), TCRB (6), COL1A2 (7) and 7C22) and one enzyme marker (PON (8)) linked to CF. By analysing the linkage relationship of these markers we are generating a genetic map of the region to provide a framework for accurate prenatal diagnosis and heterozygote detection.

The characterisation of probes closely linked to CF could help in the localisation of the gene defective in cystic fibrosis. The closer markers may provide starting points for chromosome walking (21), or probes for very large DNA fragments separated on pulse field gels (22). Candidates for the gene defective in cystic fibrosis, such as proteins involved in the β-adrenergic response or chloride ion transport, would have to map within the linkage group to be of serious interest. Finally, these closely linked probes will be invaluable in the assessment of genetic heterogeneity in cystic fibrosis.

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