Reprinted from Nature, Vol. 318, No. 6044, pp. 380-382, 28 November 1985

A polymorphic DNA marker linked to cystic fibrosis is located on chromosome 7

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Although cystic fibrosis (CF) is among the most common inherited diseases in Caucasian populations[1], the basic biochemical defect is not yet known. CF is inherited as an autosomal recessive trait apparently due to mutations in a single gene[2–4], whence the efforts made to identify the genetic locus responsible by linkage studies. Two markers have recently been identified that are genetically linked to CF: one is a genetic variation in serum level of activity of the enzyme paraoxonase[5], and the other is a restriction fragment length polymorphism (RFLP) identified with a randomly isolated DNA probe[4]. We report here that the genetic locus DOCRI-917 defined by the cloned DNA probe is located on chromosome 7.

The polymorphic locus DOCRI-917 is detected with the probe LAM4-917, a phage clone from the genomic library of Lawn *et al.*[6] which is one of a set of probes identified by a random screening procedure for RFLPs[7]. It is a 17-kilobase (kb) single-copy human genomic DNA sequence polymorphic both at a *Hin*dIII site and a *Hin*cII site in Southern blot hybridizations. The probe is frequently informative in inheritance studies, having a polymorphism information content[8] of 0.57. The structure and inheritance of the locus are described by Tsui *et al.*[4].

The marker locus DOCRI-917 was identified by DNA hybridization in rodent-human hybrid cell lines containing different human chromosome complements[9]. Hybridization of the probe to DNA from 17 hamster-human hybrids, 8 mouse-human hybrids and the parental human and rodent cell lines is shown in Fig. 1. As expected from the restriction map of the DOCRI-917 locus[4], four fragments (8.9, 5.8, 5.0 and 3.6 kb) are detected in all lanes with complementary genomic sequences. (Restriction fragment length polymorphism at the DOCRI-917 locus is not observed with *Eco*RI.) The probe does not cross-hybridize with mouse or hamster sequences (lanes CH and M).

Comparison of the LAM4-917 hybridization results with the chromosome content of the 25 cell lines indicates that the DOCRI-917 locus is situated on chromosome 7. Probe hybridization conforms to the expected pattern of a chromosome 7 locus in every line, and shows multiple discordances with all other chromosomes (Table 1). In one cell line (CH13) that scored positive with the LAM4-917 probe, chromosome 7 was present at an earlier passage but was no longer detectable by karyotype analysis or by assay of the β-glucuronidase isozyme marker GUSB[10]. At least some chromosome 7 sequences were present, however, as shown by hybridization of CH13 DNA with a complementary DNA clone of the T-cell receptor β-chain gene[11], located on chromosome 7 (q3) (refs 12–15). As shown in Table 1, the hybridization results of LAM4-917 to all 25 lines were identical to those obtained with the T-cell receptor β-chain probe.

All chromosomes except chromosome 7 are excluded because they are present in one or more of the cell line DNAs not hybridizing to LAM4-917. It is particularly important to note that chromosome 2, even though the number of discordances is low, is excluded as the location of DOCRI-917 by the absence of hybridization of the probe in the line CH8. Chromosome 2 was detected in karyotype analysis of CH8 metaphase cells, the cells were positive for the chromosome 2 isoenzymes MDH1 and IDH1[16], and hybridization to a DNA probe from chromosome 2 (D2S1[17]) is clearly detected in the same CH8 DNA sample. Exclusion of DOCRI-917 from the X and Y chromosomes was also confirmed by hybridization to a set of cell lines with sex chromosome aneuploidies (results not shown).

Because of its established genetic linkage to DOCRI-917, we conclude that the cystic fibrosis locus is also situated on chromosome 7. Determination of the location of the cystic fibrosis gene is an important step in identifying the primary genetic defect responsible for the disease. Any hypothesis that a particular genetic function is the primary lesion must satisfy the criterion of mapping to the same site as the cystic fibrosis trait. With the discovery of linkage between CF and the genetic markers PON[5] and DOCRI-917[4], the location of the disease gene is restricted to the 1% of the genome surrounding these markers. By chromosomal localization of DOCRI-917, that region is now mapped to chromosome 7. More specific localization of DOCRI-917 on

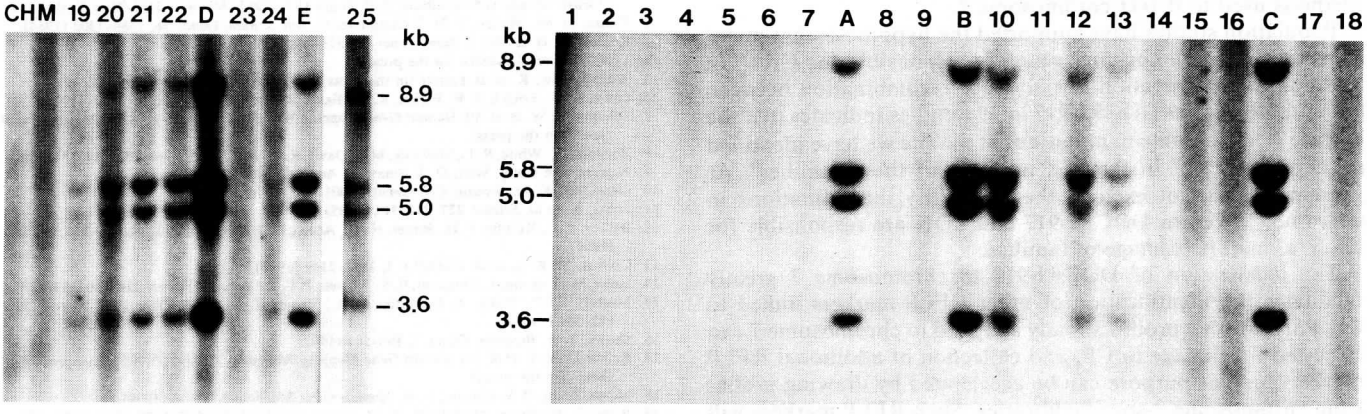

Fig. 1 Autoradiogram of hybridization of LAM4-917 to DNA of 25 somatic cell hybrids. Genomic DNA prepared from each hybrid cell line was digested with *Eco*RI, separated and transferred to DBM-filters[19]. DNA of the phage clone LAM4-917 was radioactively labelled with ^{32}P by nick-translation to a specific activity of 2×10^8 d.p.m. μg^{-1}. Hybridization was carried out for 20 h at 42 °C in 50% formamide, 0.6 M NaCl, 0.05 M Tris-HCl *p*H 7.6, 0.1% sodium pyrophosphate, 0.1% SDS, 0.04% Ficoll, 0.04% polyvinylpyrrolidone, 0.04% bovine serum albumin, 5% dextran sulphate, and 250 $\mu g\ ml^{-1}$ sonicated, denatured salmon sperm DNA. The filters were washed in 2 × SSC at 20 °C for 15 min and twice at 65 °C for 15 min, in 0.2 × SSC, 0.2% SDS at 65 °C for 40 min, and finally in 0.1 × SSC, 0.1% SDS at 65 °C for 40 min. The filters were exposed to Kodak XAR-5 film with intensifying screens (Dupont Cronex Lightning Plus) at −70 °C. The lane numbers (1–25) correspond to the hybrid cell lines listed in Table 1. Lanes 1–17 are hamster-human hybrids, and lanes 18–25 are mouse-human hybrids. A–E are the human parental cell lines, CH is the chinese hamster cell line V79.4, and M is the mouse line C11DA.

Table 1 Chromosome localization of DOCRI-917

Cell line	Human chromosomes 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	Translocation chromosomes	Hybridization TCRB	DOCRI-917
1. CH1	−	−	−	+	−	+	−	+	/	+	−	+	+	/	−	−	−	/	−	/	+	−	−	−	Xp/2q	−	−
2. CH2	−	−	+	+	−	−	−	+	−	−	−	−	−	+	−	/	−	−	/	+	−	−	−	+	Xp/2q	−	−
3. CH3	+	−	/	−	−	+	−	+	+	/	+	+	+	−	+	+	−	+	−	−	+	−	−	−	Xp/2q	−	−
4. CH4	+	−	+	+	+	/	−	+	+	−	+	+	+	+	/	−	−	+	+	+	−	−	−	−	Xp/2q	−	−
5. CH5	−	−	−	−	−	+	/	/	−	+	+	+	−	+	−	/	−	−	−	+	+	+	−	−	Xp/2q	−	−
6. CH6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	/	−	−	−	+	−	−	−	Xp/2q	−	−
7. CH7	−	−	+	+	+	−	−	+	+	−	+	+	−	+	−	+	+	−	+	+	+	−	−	−		−	−
8. CH8	+	+	+	/	+	+	−	+	+	−	−	+	−	−	−	−	−	+	+	−	−	+	−	−	Xp/5q, 5p/Xq	−	−
9. CH9	−	−	−	−	−	−	−	+	−	−	−	−	−	+	+	+	−	−	+	−	+	−	+	−		−	−
10. CH10	+	−	−	+	+	+	+	−	+	−	+	−	−	−	−	+	−	/	+	+	+	+	+	−		+	+
11. CH11	−	−	+	−	+	+	/	−	−	/	+	−	+	−	+	−	−	/	−	/	+	+	+	−	Xp/2q	−	−
12. CH12	−	−	−	+	+	+	+	/	−	−	+	+	+	+	+	+	−	+	−	+	/	+	/	−	Xp/2q	+	+
13. CH13	−	−	−	+	−	−	/*	/	−	−	+	+	−	−	−	−	−	+	+	−	−	−	+	−	Xp/2q	+	+
14. CH14	+	−	+	+	−	+	−	−	+	/	+	+	+	+	+	+	−	+	+	+	+	+	+	−	Xp/2q	−	−
15. CH15	−	−	−	+	+	+	−	/	−	+	+	−	+	+	−	/	−	−	+	−	+	−	/	−	Xp/2q	−	−
16. CH16	−	−	+	/	−	+	−	/	/	/	−	−	/	+	+	+	−	/	+	−	−	−	−	−	Xp/2q	−	−
17. CH17	−	−	−	−	−	+	−	+	−	−	+	+	−	−	−	−	−	−	−	+	/	/	+	−		−	−
18. M1	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	/	+	+	−	+	/		−	−
19. M2	+	+	−	+	+	−	+	+	−	/	+	+	−	−	+	+	+	+	+	+	+	+	/	−	Xp/5q	+	+
20. M3	−	+	+	/	+	−	+	+	−	−	−	+	+	/	−	+	+	+	−	+	+	+	−	−	5p/Xq	+	+
21. M4	/	+	+	−	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−		+	+
22. M5	−	+	+	/	+	+	+	−	−	−	+	−	−	−	−	−	+	/	+	+	+	+	/	−		+	+
23. M6	−	−	+	/	−	+	−	+	−	−	−	+	+	+	−	/	+	−	+	+	+	−	/	−		−	−
24. M7	/	/	+	+	+	/	+	+	−	−	+	−	+	/	−	−	+	−	+	/	−	−	/	−		+	+
25. M8	−	+	+	−	−	+	+	−	−	−	+	+	−	−	−	−	+	−	−	−	/	−	+	−	Xp/5q, 5p/Xq	+	+
Discordant DOCRI-917 (%)	39	17	50	40	28	57	0	60	57	55	36	48	50	68	54	43	21	29	48	45	59	29	32			0	

Chromosomal content of hybrid cell lines was determined by cytogenetic and isoenzyme analysis. The panel of hybrid cell lines was generated and characterized by Nguyen Van Cong, Dominique Weil and Catherine Finaz, Unité de Recherches de Génétique Médicale, INSERM U12, Paris, France, and is described elsewhere[9]. Symbols: +, chromosome detected in more than 30% of cells; −, chromosome not detected; /, chromosome detected in less than 30% of cells, not scored for mapping. Three of the human parental cell lines contained reciprocal chromosomal translocations[20,21] retained by some hybrids: Xq/2q (Xqter→p22:2q32→2qter), Xp/5q (Xpter→q21:5q11→qter), and 5p/Xq (5pter→q11:Xq21→qter). Hybridization results for DOCRI-917 are from the autoradiogram in Fig. 1. The TCRB gene is detected by hybridization with the T-cell receptor β-chain probe[11] with the same cellular DNA samples. * Possible chromosome rearrangement, see text.

chromosome 7 will be possible by testing other hybrid cell lines containing partial deletions of chromosome 7, *in situ* hybridization to metaphase chromosomes, and linkage mapping with other chromosome 7 markers. The observation that the DOCRI-917 locus is present in cell line CH13, which appears to retain only a part of chromosome 7 lacking GUSB (cen→q22) (ref. 18) but retaining the T-cell receptor β-chain gene TCRB on 7q3 is consistent with a location on 7q, but other explanations of this result are possible. For example, more than one segment of chromosome 7 might have segregated with this line, or the DNA hybridization may simply be more sensitive than the other methods used to detect chromosome 7.

Population studies have supported the hypothesis that mutations in a single autosomal gene are responsible for CF[2,3]. In addition, the observation of only 15% recombination between CF and the marker DOCRI-917 in 39 families indicates that the disease is attributable to mutations at the site we have identified on chromosome 7 in the vast majority of these families[4]. At present, we cannot exclude the possibility that mutations in genes not linked to DOCRI-917 and PON are responsible for CF in a small percentage of families.

The localization of DOCRI-917 to chromosome 7 greatly facilitates the identification of other RFLP markers linked to CF. Polymorphic probes already assigned to chromosome 7 can be tested for linkage to CF, and collection of additional RFLP markers for this purpose can be accelerated by drawing probes from chromosome 7-specific libraries. Such RFLP markers will contribute to increasing the resolution of the linkage map of the CF region, and ultimately to the identification of the CF gene itself.

We thank Marie-France de Tand, Chantal Foubert and Marie-Sylvie Gross for their technical assistance.

Note added in proof: Our recent linkage analysis shows that both CF and DOCRI-917 are closely linked to the proα2(1) collagen gene (7q21-q22) (ref. 22 N. Buchwald et al., in preparation).

Received 11 November; accepted 12 November 1985.

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Printed in Great Britain by Macmillan Production Ltd., Basingstoke, Hampshire