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Identification and Regional Localization of DNA Markers on Chromosome 7 for the Cloning of the Cystic Fibrosis Gene

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Summary

To facilitate mapping of the cystic fibrosis locus (*CF*) and to isolate the corresponding gene, we have screened a flow-sorted chromosome 7–specific library for additional DNA markers in the 7q31-q32 region. Unique ("single-copy") DNA segments were selected from the library and used in hybridization analysis with a panel of somatic cell hybrids containing various portions of human chromosome 7 and patient cell lines with deletion of this chromosome. A total of 258 chromosome 7–specific single-copy DNA segments were identified, and most of them localized to subregions. Fifty three of these corresponded to DNA sequences in the 7q31-q32 region. Family and physical mapping studies showed that two of the DNA markers, *D7S122* and *D7S340*, are in close linkage with *CF*. The data also showed that *D7S122* and *D7S340* map between *MET* and *D7S8*, the two genetic markers known to be on opposite sides of *CF*. The study thus reaffirms the general strategy in approaching a disease locus on the basis of chromosome location.

Introduction

"Reverse genetics" has become a widely used approach in studying diseases with poorly defined biochemical defects (reviewed in Orkin 1986). The general strategy involves the application of classical genetic linkage analysis and cytogenetic analysis with a number of well-localized DNA markers to reveal the chromosomal location of the disease locus. Subsequent cloning and examination of the DNA sequences in the region allows the isolation of candidate genes which can be tested for their involvement in the disease. Recent successes with this approach include the cloning of the genes responsible for Duchenne muscular dystrophy (Monaco et al. 1986; Burghes et al. 1987), chronic granulomatous disease (Royer-Pokora et al. 1986), and retinoblastoma (Friend et al. 1986).

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Cystic fibrosis (CF) is one of the major genetic diseases whose primary lesion has been elusive, largely because of the difficulty in deriving the basic biochemical defect from the complex clinical symptoms. Patients with CF suffer from chronic obstructive pulmonary diseases, pancreatic enzyme insufficiency, and malfunctions in other exocrine glands, including an elevated electrolyte content in the sweat (reviewed in Talamo et al. 1983). Recent studies have shown that the underlying cause of this autosomal recessive disorder involves the abnormal regulation of chloride ion transport in secretory epithelial cells (Frizzell et al. 1986; Welsh and Liedtke 1986; Schoumacher et al. 1987; Li et al. 1988). Since there is only a limited understanding of the molecular basis of this regulation, it has not yet been possible to pinpoint the basic defect on the basis of these studies.

Extensive genetic linkage analysis has, however, allowed mapping of the CF locus (*CF*) to the long arm of chromosome 7 (reviewed in Tsui et al. 1986*b*). The genetic markers that have been found linked to *CF* include *PON* (Eiberg et al. 1985), D7S15 (917) (Knowlton et al. 1985; Tsui et al. 1985), *COL1A2* (Scambler et

al. 1985; Buchwald et al. 1986), *MET* (White et al. 1985), *D7S8* (J3.11) (Wainwright et al. 1985), *TCRB* (Wainwright et al. 1985), *D7S18* (7C22; formerly *D7S16*) (Scambler et al. 1986*b*), *D7S13* (B79a) (Wainwright et al. 1987), and the recently identified *D7S23* (XV2C) (Estivill et al. 1987). The order of these markers has also been established: *centromere-COL1A2-D7S15-(PON-D7S13)-D7S18-MET-(D7S23*–CF)-D7S8-TCRB-telomere (Beaudet et al. 1986; Barker et al. 1987*a*; Lathrop et al. 1988; Poustka and Bates 1988). The relative order of *D7S23* and *CF* has only been suggested in one report (Berger et al. 1987), and that of *PON* and *D7S13* has not been established. Since the two markers that closely flank *CF–MET* and *D7S8*–have been localized to band 7q31 (Dean et al. 1985; Zengerling et al. 1987; Park et al. 1988; Spence and Tsui 1988), it is probable that *CF* also resides in the same region. The distance between them and *CF* has been estimated to be probably less <0.5 centimorgans (cM) (Beaudet et al. 1986; Lathrop et al. 1988).

Our laboratory has engaged in the isolation of additional DNA segments from the 7q31-q32 region for the identification of markers closer to *CF* and for the cloning of the corresponding gene. Our basic strategy has been to isolate a large number of DNA markers from the q31 region and to identify those closest to *CF* by using both genetic and physical mapping methods. On the basis of its size relative to that of the total genome (Morton et al. 1982), chromosome 7 should contain ~150 million bp (Mb) of DNA, 20% of which should be within bands q31-q32. Since *MET* and *D7S8* have been estimated to be one to several cM (or Mb) apart (Barker et al. 1987*a*; Lathrop et al. 1988), we reasoned that we should be able to identify one or more markers between *MET* and *D7S8*, and therefore closer to *CF*, by screening 50 random DNA segments from the q31-q32 region. It would then be possible to use these closely linked markers in chromosome walking and jumping experiments to clone the entire region containing the CF gene.

The present report documents the isolation and characterization of 258 markers on chromosome 7, 53 of which are located in the q31-q32 region. Thus far, our analyses have shown that two of the markers are located between *MET* and *D7S8* and are closely linked to *CF*.

Material and Methods

Source of DNA Probes

The DNA segments (probes) used in the present study were isolated from a chromosome 7–specific library constructed by the Los Alamos and Lawrence Livermore Laboratories. The library, prepared in λCharon 21A vector with *Eco*RI-digested DNA from flow-sorted chromosome 7 (from the hamster-human somatic cell hybrid line MR3.31–6TG6) (Deaven et al. 1986), was obtained through the American Type Culture Collection, Rockville, MD. Two different methods were used in selecting the probes. First, a total of 2,268 phage plaques were picked and propagated in *Escherichia coli* K803 by standard procedures (Maniatis et al. 1982). Ordered arrays of phage plaques were transferred to nitrocellulose filters in duplicate sets (Benton and Davis 1977) to permit identification of clones containing repetitive DNA sequences. Some of the DNA probes that were useful in subsequent studies were subcloned into plasmid vector pUC9, pUC13, pGEM3, pBR322, or pSV2-gpt.

In the second method, the recombinant DNA inserts of ~1 × 10^6 plaque-forming units from the chromosome 7 library were purified from the phage arms by agarose-gel electrophoresis and "shotgun" cloned into the *Eco*RI site of the plasmid vector pUC13. More than 10,000 colonies were obtained, and approximately 2,000 were selected for further analysis. The plasmid-containing bacterial cultures were streaked on agar plates in organized arrays and transferred to nitrocellulose filters for examination of repetitive DNA sequences as described below.

Screening for Repetitive DNA Sequences

Nitrocellulose filters containing arrays of recombinant phages or plasmids were hybridized with radioactively labeled total human or hamster DNAs in order to identify those clones that were free of repetitive DNA sequences. Total human and hamster DNAs were prepared from human placenta (Sigma) and Chinese hamster ovary cells, respectively, and were labeled with [α^{32}P]-dCTP by nick-translation (Rigby et al. 1977).

Probe Preparation for DNA Blot Hybridization

Phage DNA stocks were prepared from agarose plates according to procedures described elsewhere (Maniatis et al. 1982), and 10 mM $MgSO_4$ was supplemented in the medium. An average of 5 μg of phage DNA were obtained per plate. Plasmid DNA stocks were prepared from liquid cultures (3 ml) by the modified boiling procedure of Holmes and Quigley (1981) or the alkaline lysis method of Birnholm (1983). The recombinant (human) DNA inserts were purified from the individually amplified phage or plasmid stocks by *Eco*RI digestion and electroelution from agarose gels. The probes were

labeled with [α^{32}P]-dCTP by the random priming method (Feinberg and Vogelstein 1983).

Somatic Cell Lines

The somatic cell hybrid lines used for DNA marker mapping studies are summarized in figures 1*A* and 1*B*. The human/mouse hybrids carrying a whole or some part of human chromosome 7 (5387 3 cl 10, RuRag 6-19, Ru-Rag 4-13, Ru-Rag 1a-5-11, MH-Rag 8-7, IT-9a 2-21-14, 194-Rag 6-13, or IT-A9 X-28-13), patient cell lines carrying deleted chromosome 7 (GM3240, GM3733, or GM1059), and human fibroblast cell lines with two normal chromosome 7's (GM3098) have been described elsewhere (Zengerling et al. 1987). The hamster/human somatic cell hybrids 4AF1/106/K015, 1CF2/5/K016, and 1EF2/3/KO17 are subclones of 4AF1/106, 1CF2/5, and IEF2/3, respectively, which were originally described by Arfin et al. (1983). Karyotype analysis and nucleic acid hybridization studies with DNA markers of known chromosomal locations revealed that 4AF1/106/KO15 is essentially as previously reported. However, 1EF2/3 and two of the subclones, 1CF2/5/KO16 and 1EF2/3/KO17, had apparently suffered further rearrangements (data not shown); the human chromosome 7 materials retained in these cell lines are shown in figure 1*B*. The human/mouse hybrid GM1059-Rag5 contains the chromosome 7 with an interstitial deletion from GM1059 (7pter-q22::q32-qter), and 2068Rag22–2 contains the translocated chromosome t (6,7) (6pter-q27::7q22-qter) (K.-H. Grzeschik, personal communication).

RFLP Analysis

Restriction-enzyme digestions of DNA samples (from hybrid cell lines and CF family members) were performed under specifications recommended by the suppliers. Agarose-gel electrophoresis and blot-hybridization analysis have been described elsewhere (Tsui et al. 1985; 1986*b*; Buchwald et al. 1986). RFLPs were identified by gel-blot hybridization analysis of random human DNA samples digested with 20–30 commonly used enzymes. The RFLPs were then examined in a subset of CF families in which recombination events were noted in the vicinity of *CF*. If the probe was found to

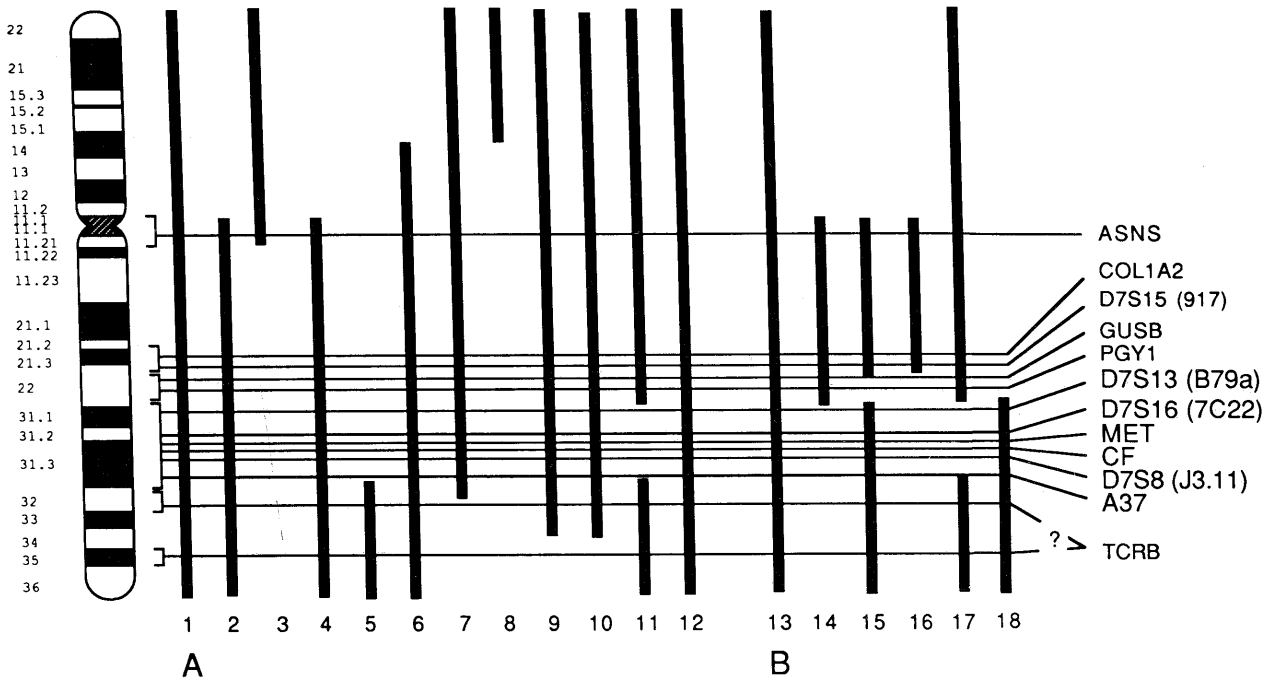

Figure 1 Somatic cell hybrids used in the present study. A schematic diagram of human chromosome 7 is shown on the left. Vertical lines represent the amounts of diagnostic human chromosome 7 present in each cell line. *A*, Human/mouse somatic cell hybrids (lane 1, 5387 3 cl 10; lane 2, RuRag 6-19; lane 3, Ru-Rag 4-13; lane 4, Ru-Rag 1a-5-11; lane 5, MH-Rag 8-7; lane 6, IT-9a 2-21-14; lane 7, 194-Rag 6-13; lane 8, IT-A9 X-28-13), patient cell lines carrying deleted chromosome 7 (lane 9, GM3240; lane 10, GM3733; lane 11, GM1059), and human fibroblast cell line with two normal chromosome 7's (lane 12, GM3098) (details in Zengerling et al. 1987); *B*, Hamster/human somatic cell hybrids (lane 13, 4AF1/106/KO15; lane 14, 1CF2/5/KO16; lane 15, 1EF2/3; lane 16, 1EF2/3/KO17) and human/mouse hybrids (lane 17, GM1059-Rag 5; lane 18, 2068Rag22-2). The regional assignments of the genetic markers shown on the right are derived from a previous study (Zengerling et al. 1987) and the present study.

be close to *CF*, additional families were analyzed. DNA marker haplotypes for the normal and CF chromosomes were obtained from the parental chromosomes by inspection.

Linkage Analysis

The maximal likelihood estimate for recombination fraction (θ) between two genetic loci was obtained by pairwise linkage analysis using the LIPED computer program (Ott 1974). The CF families used in the present study have been described elsewhere (Tsui et al. 1986*b*; White et al. 1986). Linkage disequilibrium was calculated according to the method of Chakravarti et al. (1984).

Pulsed-Field Gel Electrophoresis

The DNA samples for pulsed-field gel electrophoresis were prepared from lymphoblast line HSC55 (data not shown) embedded in low-melting-point agarose (Seakem) essentially according to the method of Van Ommen and Verkerk (1986). Restriction-enzyme digestions were carried out with 1–5 units enzyme/μg DNA in 5 vol appropriate buffer under conditions recommended by the enzyme manufacturer. Electrophoretic separation of large DNA fragments was performed in a "cross-field" gel apparatus as described by Southern et al. (1987).

Results

Library Screening

Two major screening efforts were conducted to identify DNA markers useful for *CF* linkage analysis. In the first screening, the flow-sorted chromosome 7 phage library was plated at low density and more than 2,000 clones were picked. Phage spots were transferred to nitrocellulose and hybridized with radioactively labeled total human DNA in order to identify those clones that did not contain any repetitive DNA elements and that therefore were suitable for probe hybridization analysis. Since a small amount of hamster chromosome material could have been copurified with human chromosome 7 during flow sorting, labeled total Chinese-hamster-ovary DNA was also used as probe to identify sequences that were of hamster origin. The result is summarized in table 1.

Two major technical difficulties were experienced during the first screening. First, a significant fraction (14%) of the phage isolates failed to produce plaques on respotting, presumably owing to poor recovery from the primary plating. Second, both the preparation of large quantities of phage lysates and the electroelution of the inserts from these phage stocks were extremely tedious. To circumvent these problems, phage inserts were first subcloned into a plasmid vector before the second screening was performed. This modification greatly reduced the time and effort involved. Almost all (99.8%) of the plasmids recovered contained inserts >1 kb in size. In addition, a higher proportion of single-copy clones was recovered (table 1). The significance of the latter observation has not been followed up, but it is possible that the subcloning step might have selected against clones that contained highly repetitive DNA sequences.

From the two screenings, a total of 1,757 clones were found to be free of highly repetitive DNA sequences. Since a large percentage (>20%) of the clones containing repeat sequences were found to be of hamster origin, it seemed probable that a similar proportion of the single-copy clones might also be derived from hamster sequences. The only simple method to identify clones of the latter class was to test for their ability

Table 1

Summary of Probe Screening

Clones Isolated (*N*)	Human/Hamster Repeats	Picked and Analyzed	Mapped on 7	Probes within 7q31-q32
I[a] (2,268)	1,446	255	118	12
II[b] (1,724).....	789	241	140	41
Total (3,992)	2,235	496	258	53

[a] DNA segments were individually purified from phage clones and used as probes in hybridization analysis; the majority of the probes from the 7q31-q32 region were initially identified by half-intensity hybridization signal with GM1059 (see fig. 1A).

[b] DNA segments from a chromosome 7–specific library were subcloned into plasmid vector pUC13 in a pool; individual inserts from the plasmids were used in hybridization analysis; all probes from the 7q31-q32 region were identified with the use of the somatic cell hybrid panel shown in fig. 1B.

to detect hamster sequences by gel blot–hybridization analysis. Hence, this step was combined with the regional localization studies (see below). Of the 496 probes studied, 198 (40%) were found to be hamster specific and thus were eliminated from further characterization. In the same analysis, 39 of the remaining clones were found to contain moderately repetitive human-specific sequences and were also excluded. Only 258 clones (52%) were found to be bona fide human-specific single-copy probes. The size range of these *Eco*RI fragments was 0.5–6.5 kb, and the average was 3.3 kb (see table 2). The unique identities of these DNA segments were established by analysis with additional restriction-enzyme digestions (data not shown).

Regional Localization

Regional localization of DNA markers was accomplished by blot-hybridization analysis with the aid of both somatic cell hybrids carrying various portions of human chromosome 7 and cell lines with chromosome 7 deletions. Since additional, more informative somatic cell hybrids and cell lines became available at various points during the course of our work, the screening process was performed in three stages.

Screening was initially performed with a set of mouse-human hybrid cell lines and three patient cell lines with deletions spanning the distal half of 7q (see fig. 1*A*). This set of hybrids was used successfully to localize a number of DNA markers closely linked to *CF* (Zengerling et al. 1987). The most informative cell line was GM1059, which contained a single interstitial deletion spanning the *CF* region (46,XX; del7 (pter-q22::q32-qter)). We showed that all the DNA markers closely linked to *CF* were hemizygous in this cell line (Zengerling et al. 1987). Of the 76 human-specific single-copy probes screened with this set of cell lines, only two were found to be located within the region deleted in GM1059. These clones have been described in a preliminary report (Tsui et al. 1987). It is possible that some of the clones from the q22–32 region were inadvertently missed at this stage, because identification of probes within the CF region required careful densitometric comparison of the hybridization signals in GM1059 with those in cell lines containing two copies of 7q22-q32.

To improve the efficiency in identifying clones in the q31-q32 region, a set of human-hamster hybrids containing a whole or parts of chromosome 7 as the only human material (data not shown) was included in the probe-hybridization analysis in the second stage of screening. The amount of human chromosome 7 retained in each of these cell lines is shown in figure 1*B*. Of 38 probes analyzed at this stage, 11 revealed hybridization signals in lines KO15 and KO17 but not in KO16; six were subsequently shown to be within the deletion in GM1059 (Tsui et al. 1987).

The final stage of our screening effort was greatly facilitated by the availability of a mouse-human somatic cell hybrid 1059Rag5 (K.-H. Grzeschik, personal communication), which contains the del7 chromosome of GM1059. This cell line, together with KO15, KO16, KO17, and rag22–2 (a mouse-human cell line containing 7q22-qter [K.-H. Grzeschik, personal communication]), was used to identify the remaining 45 DNA probes mapped to the 7q31-q32 region. Examples of hybridization results are shown in figure 2.

The regional localization of the 258 DNA segments on chromosome 7 derived from all three stages of screening is shown in figure 3. A brief description of these chromosome 7–specific probes is summarized in table 2. Fifty-three of the 258 unique DNA segments are located in the 7q31-q32 region, and hence this number is in perfect agreement with the estimated size of this region.

Identification of DNA Polymorphisms

We next attempted to identify RFLPs associated with each of the potentially interesting marker loci for use in CF linkage analysis. The screening was carried out by gel blot–hybridization analysis of DNA samples from 5–10 random individuals and/or *CF* parents. A variety of commonly used restriction enzymes were used. The RFLPs identified in this study are listed in table 3. As shown in the table, the majority of RFLPs recognized by the 43 probes are simple, two-allele systems with an average PIC value of 0.3 (Botstein et al. 1980).

Linkage Analysis with CF

Of the 53 DNA markers that were localized to 7q31-q32, 21 were found to associate with detectable RFLPs (see table 3). Linkage analysis was performed to determine the relationships between these polymorphic markers and *CF*. Each marker was first tested with a set of families in which recombination breakpoints were documented between *MET* and *CF* (Tsui et al. 1986*a*) or between *D7S8* and *CF* (White et al. 1986). If no recombination with *CF* was noted, the marker was further examined by using the other CF families in our collection (Tsui et al. 1986*b*). As a result, most of the markers were excluded from further analysis because of the detection of recombination events between the

Table 2

List of 258 DNA Probes on Chromosome 7

Name	Vector	Insert[a] (kb)	HGM No.[b]	Location[c]	RFLP[d]
pA-6	pUC13	3.7	D7S 155	7q31-q32	
pA-12	pUC13	2.9	D7S 156	7p	
pA-14	pUC13	2.5	D7S 157	7p	
pA-21	pUC13	3.6	D7S 114	7q32-qter	+
pA-33	pUC13	2.5	D7S 158	7p11-q22	
pA-35	pUC13	4.0	D7S 159	7q22	
pA-37	pUC13	4.3	D7S 115	7q31-q32	+
pA-44	pUC13	3.2	D7S 160	7p?	
pA-45	pUC13	3.3	D7S 161	7q22	
pA-46	pUC13	4.2	D7S 162	7q32-qter	
pA-51	pUC13	3.7	D7S 116	7q31-q32	+
pA-75	pUC13	1.6	D7S 163	7q31-q32	
pA-77.1	pUC13	3.8	D7S 164	7q31-q32?	
pA-85	pUC13	4.1	D7S 165	7q22	
pA-88	pUC13	2.0	D7S 166	7q22	
pA-89	pUC13	3.4	D7S 167	7p?	
pA-93	pUC13	4	D7S 168	7q32-qter	
pA-94	pUC13	1	D7S 169	7p11-q22	
pA-100	pUC13	4.5	D7S 170	7p11-q22	
pA-116	pUC13	4.5	D7S 171	7p?	
pA-117	pUC13	4.5	D7S 172	7p?	
pA-130	pUC13	3.0	D7S 173	7q32-qter	
pA-146	pUC13	2.9	D7S 174	7p11-q22	
pA-152	pUC13	2.0	D7S 175	7p11-q22	
pA-160	pUC13	2.0	D7S 176	7p11-q22	
pA-164	pUC13	2.0	D7S 177	7q22	
pA-165	pUC13	4.2	D7S 178	7q22	
pA-171L	pUC13	2.0	D7S 179	7p11-q22	
pA-187	pUC13	3.7	D7S 180	7q31-q32	
pB-4	pUC13	3.7	D7S 181	7p	
pB-16	pUC13	3.6	D7S 182	7q32-qter	
pB-36	pUC13	2.2	D7S 393	7p?	
pB-37	pUC13	1.6	D7S 183	7q22	
pB-47L	pUC13	3.7	D7S 391	7q31-q32	+
pB-48	pUC13	1.3	D7S 117	7q31-q32	+
pB-49	pUC13	3.2	D7S 185	7p11-q22	
pB-68L	pUC13	1.6	D7S 186	7q22	
pB-69L	pUC13	1.5	D7S 187	7p?	
pB-69U	pUC13	2.0	D7S 188	7p?	
pB-83	pUC13	4.0	D7S 189	7p	
pB-85L	pUC13	3.2	D7S 190	7p	
pB-88L	pUC13	3.6	D7S 191	7q32-qter	
pB-112	pUC13	3.9	D7S 192	7p11-q22	
pB-117.2	pUC13	5.4	D7S 193	7q31-q32	
pB-119	pUC13	3.3	D7S 194	7p11-q22	
pB-121L	pUC13	4.0	D7S 195	7q22	
pB-130	pUC13	2.7	D7S 118	7q31-q32	+
pB-140	pUC13	3.8	D7S 196	7q31-q32	
pB-171	pUC13	1.1	D7S 197	7q32-qter	
pB-173	pUC13	1.6	D7S 198	7p11-q22	
pB-174	pUC13	5.1	D7S 119	7q31-q32	+
pB-177	pUC13	1.2	D7S 199	7q31-q32	
pB-178	pUC13	5.0	D7S 200	7q31-q32	
pB-189	pUC13	3.7	D7S 201	7q31-q32	+

(*continued*)

Table 2 (continued)

Name	Vector	Insert[a] (kb)	HGM No.[b]	Location[c]	RFLP[d]
pB-192	pUC13	3.7	D7S 120	7q31-q32	+
pC-67.1	pUC13	2.4	D7S 202	7q31-q32	+
pC-82	pUC13	1.5	D7S 203	7q32-qter	
pC-96	pUC13	4.2	D7S 204	7q32-qter	
pC-100	pUC13	3.1	D7S 205	7q22	
pC-102	pUC13	2.2	D7S 206	7q31-q32	+
pC-105	pUC13	3.3	D7S 207	7p?	
pC-111U	pUC13	3.1	D7S 208	7p?	
pC-123	pUC13	.5	D7S 209	7p11-q22	
pC-129	pUC13	4.5	D7S 210	7p11-q22	
pC-133	pUC13	4.4	D7S 211	7q31-q32	
pC-140	pUC13	2.8	D7S 212	7p	
pC-148L	pUC13	3.5	D7S 213	7q32-qter	
pC-149	pUC13	3.1	D7S 214	7q32-qter	
pC-158	pUC13	1.8	D7S 215	7p11-q22	
pC-162	pUC13	3.5	D7S 216	7q31-q32	
pD-35	pUC13	3.2	D7S 217	7q22	
pD-37	pUC13	3.3	D7S 218	7p11-q22	
pD-44L	pUC13	3.5	D7S 219	7q22	
pD-50	pUC13	2.1	D7S 220	7q32-qter	
pD-54	pUC13	3.6	D7S 221	7p11-q22	
pD-102U	pUC13	3.5	D7S 222	7q31-q32	
pE-52	pUC13	3.5	D7S 223	7p11-q22	
pE-62	pUC13	3.6	D7S 224	7p11-q22	
pE-65U	pUC13	3.2	D7S 225	7p11-q22	
pE-69L	pUC13	2.1	D7S 226	7q	
pE-77	pUC13	3.4	D7S 227	7p11-q22	
pE-79	pUC13	5.7	D7S 228	7q32-qter	+
pE-80	pUC13	4.5	D7S 229	7q22	
pE-115	pUC13	2.4	D7S 230	7p11-cen?	
pE-128.1	pUC13	2.1	D7S 231	7q31-q32	
pE-128U	pUC13	3.3	D7S 232	7p11-q22	
pE-130	pUC13	2.6	D7S 233	7q31-q32	
pE-146L	pUC13	2.5	D7S 234	7p	
pE-146U	pUC13	3.6	D7S 235	7p11-q22	
pE-159	pUC13	6.2	D7S 236	7q22	
pE-168	pUC13	1.0	D7S 237	7q32-qter	
pE-170	pUC13	2.1	D7S 238	7q22	
pF-14	pUC13	2.6	D7S 239	7p11-q22	
pF-16	pUC13	3.5	D7S 240	7q22	
pF-22L	pUC13	3.5	D7S 241	7p11-q22	
pF-37	pUC13	2.0	D7S 242	7p11-q22	
pF-43	pUC13	4.0	D7S 243	7p11-q22	
pF-49	pUC13	6.0	D7S 244	7q22	
pF-122	pUC13	2.0	D7S 245	7q22	
pF-140	pUC13	4.7	D7S 246	7q32-qter	
pF-141.1	pUC13	3.6	D7S 121	7q31-q32	
pF-141U	pUC13	3.9	D7S 247	7q32-qter	
pF-153	pUC13	3.6	D7S 248	7q32-qter	
pF-156	pUC13	4.2	D7S 249	7p11-q22	
pF-157	pUC13	3.4	D7S 250	7q32-qter	
pF-159L	pUC13	2.3	D7S 251	7q32-qter	
pF-167.2	pUC13	5.8	D7S 252	7q31-q32	+
pF-175	pUC13	2.7	D7S 253	7q32-qter	

(*continued*)

Table 2 (continued)

Name	Vector	Insert[a] (kb)	HGM No.[b]	Location[c]	RFLP[d]
pF-179.3	pUC13	5.2	D7S 254	7q31-q32	
pF-179M	pUC13	3.9	D7S 255	7q22	
pG-2	pUC13	3.2	D7S 256	7p11-q22	
pG-8	pUC13	3.0	D7S 257	7p11-q22	
pG-54	pUC13	4.0	D7S 258	7q32-qter	+
pG-58U	pUC13	5.4	D7S 260	7q22	
pG-120U	pUC13	2.1	D7S 261	7p11-q22	
pG-122L	pUC13	4.3	D7S 262	7q22	
pG-139L	pUC13	1.0	D7S 263	7q32-qter	
pG-144	pUC13	6.0	D7S 264	7q22	
pG-146	pUC13	2.2	D7S 265	7p11-q22	
pG-153	pUC13	5	D7S 266	7q22	
pG-163	pUC13	5.5	D7S 267	7p11-q22	
pG-166	pUC13	3.0	D7S 268	7q22	
pG-171	pUC13	2.1	D7S 269	7q22	
pG-172	pUC13	1.9	D7S 270	7q32-qter	
pG-173	pUC13	4.7	D7S 271	7q32-qter	
pG-176U	pUC13	3.5	D7S 272	7p11-q22	
pG-179	pUC13	2.3	D7S 273	7p	
pH-131	pUC13	3.4	D7S 122	7q31-q32	+
pH-140	pUC13	2.8	D7S 274	7p11-q22	
pJB85B	pUC13	2.3	D7S 151	7q31-q32	
pJB89	pUC13	1.2	D7S 152	7q31-q32	+
pJB101	pUC13	6.0	D7S 153	7q31-q32	
pJB117	pUC13	2.9	D7S 154	7q31-q32	
pJB5-21	pUC13	6.0	D7S 113	7q31-q32	+
pH-165	pUC13	2.4	D7S 275	7p11-q22	
pH-172L	pUC13	5.6	D7S 276	7q32-qter	
pH-176	pUC13	2.4	D7S 277	7p11-q22	
pH-191	pUC13	4.4	D7S 278	7q31-q32	
pI-1L	pUC13	3.6	D7S 279	7q32-qter	
pI-54	pUC13	1.3	D7S 280	7q31-q32?	
pI-56	pUC13	1.3	D7S 281	7q22	
pI-64	pUC13	3.1	D7S 282	7q32-qter	
pI-65	pUC13	3.8	D7S 283	7q31-q32?	+
pI-92	pUC13	5.0	D7S 284	7q32-qter	
pI-98	pUC13	2.1	D7S 285	7q31-q32	
pI-113	pUC13	3.5	D7S 286	7p11-q22	
pI-114U	pUC13	4.6	D7S 287	7p11-q22	
pI-115U	pUC13	4.4	D7S 288	7q32-qter	
pJ-16	pUC13	4.2	D7S 123	7q31-q32	
pJ-18	pUC13	2.1	D7S 124	7q31-q32	+
pJ-31	pUC13	1.3	D7S 289	7q22	
pJ-43	pUC13	3.1	D7S 290	7q31-q32	
pJ-62	pUC13	1.0	D7S 291	7q22	
pJ-71	pUC13	3.9	D7S 292	7q32-qter	
pJ-74	pUC13	4.1	D7S 293	7q22	
pJ-76	pUC13	3.5	D7S 294	7q32-qter	
SA5	pUC9	4.8	D7S 295	7q31-q32?	
SA12	Ch21A	4	D7S 296	7q32-qter	
SA34	pUC9	3.4	D7S 297	7q31-q32?	
SA36	Ch21A	2.2	D7S 298	7cen-q22	
SA37	pUC13	4.2	D7S 125	7q31-q32	+
SA39	Ch21A	2	D7S 299	7p	
SB56	Ch21A	3.6	D7S 300	7cen-q22	

(*continued*)

Table 2 (continued)

Name	Vector	Insert[a] (kb)	HGM No.[b]	Location[c]	RFLP[d]
SB61	Ch21A	.7	D7S 301	7cen-q22	
SB69U	Ch21A	1.6	D7S 302	7p	
SC33	pUC9	3.5	D7S 126	7q31-q32	+
SC34	Ch21A	4.0	D7S 303	7cen-q22	
SC42	Ch21A	1.7	D7S 304	7cen-q22	
SC55	pUC9	1.5	D7S 305	7q31-q32	
SC56	Ch21A	3.6	D7S 306	7q32-qter	
SC69	Ch21A	3.3	D7S 307	7q32-qter	
SD6	Ch21A	3.2	D7S 308	7q32-qter	
SD31	Ch21A	3.5	D7S 309	7cen-q22	
SD37	Ch21A	5.2	D7S 310	7q32-qter	
SD40	Ch21A	4.8	D7S 311	7q32-qter?	
SD49	Ch21A	3.5	D7S 312	7cen-q22	
SE4	Ch21A	4.0	D7S 313	7q32-qter	
SE44	Ch21A	2.9	D7S 314	7p	
SF4	Ch21A	4.2	D7S 315	7pter-p14	
SF32	pUC9	3.5	D7S 316	7q31-q32	+
SF35L	pUC13	3.2	D7S 317	7q31-q32	
SF60	Ch21A	2.9	D7S 318	7cen-q22	
SI32	pGem3	2.9	D7S 127	7q31-q32	+
SI36	Ch21A	2.2	D7S 319	7cen-q22	
SI50	Ch21A	5.1	D7S 320	7q32-qter	
SJ51	Ch21A	1.7	D7S 321	7cen-q22	
TD52	pSV2-gpt	4.7	D7S 128	7pter-p14	+
TD125	pSV2-gpt	2.0	D7S 322		
TD145	pSV2-gpt	4.0	D7S 323		
TG16	pSV2-gpt	2.2	D7S 129	7cen-q22	+
TG20	pSV2-gpt	3.3	D7S 324		
TG38	pSV2-gpt	2.4	D7S 325	7p14-cen	
TG43	Ch21A	4.5	D7S 326	7cen-q22	
TG81	pBR322	1.2	D7S 327		
TG83	pSV2-gpt	3.2	D7S 328	7p11-q11	
TG96	pSV2-gpt	4.5	D7S 329		
TG104	pSV2-gpt	3.1	D7S 330	7p11-q11	
TG105	pSV2-gpt	3.3	D7S 130	7q32-q34	+
TG106	pSV2-gpt	3.0	D7S 331	7cen-q22	
TG108	pSV2-gpt	2.3	D7S 332		
TG121	pSV2-gpt	2.5	D7S 333	7pter-p14	
TG126.2	pUC13	.85	D7S 334	7q31-q32	
TG137	pSV2-gpt	2.5	D7S 131		
TG160	Ch21A	2.6	D7S 335	7cen-q22	
TG175	pSV2-gpt	3.1	D7S 336	7cen-q22	
TM13	pSV2-gpt	2.0	D7S 337		
TM17	pSV2-gpt	.5	D7S 338	7q31-q32	
TM54	pSV2-gpt	4.0	D7S 339	7p14-cen	
TM58.2	pUC13	3.3	D7S 340	7q31-q32	
TM60	pSV2-gpt	2.3	D7S 132	7pter-p14	+
TM62	pUC9	1.8	D7S 133		
TM66L	pSV2-gpt	1.3	D7S 341	7cen-q22	
TM66U	pSV2-gpt	2.5	D7S 342	7pter-p14	
TM70	Ch21A	2.1	D7S 343		
TM77	Ch21A	3.2	D7S 134	7pter-p14	+
TM99/98U	pSV2-gpt	3.9	D7S 344	7p11-q11	
TM102L	pSV2-gpt	1.7	D7S 135	7pter-p14	+
TM102U	pSV2-gpt	2.0	D7S 345		

(*continued*)

Table 2 (continued)

Name	Vector	Insert[a] (kb)	HGM No.[b]	Location[c]	RFLP[d]
TM138L	pBR322	1.9	D7S 136		+
TM178	pSV2-gpt	2.1	D7S 137		+
TM182	pSV2-gpt	3.0	D7S 346		
TM183	pSV2-gpt	3.8	D7S 347	7q31-q32	+
TM196	pSV2-gpt	3.3	D7S 392	7q34-qter	+
TN3	pSV2-gpt	1.7	D7S 394		
TN11	pUC9	2.0	D7S 139		+
TN27	pSV2-gpt	1.7	D7S 140	7q31-q32	
TN31	pSV2-gpt	2.5	D7S 348		
TN34L	pBR322	2.1	D7S 349	7p14-cen	
TN36	Ch21A	4.0	D7S 350		
TN56	pBR322	6.5	D7S 141		+
TN66	Ch21A	8	D7S 142	7pter-p14	+
TN68	pUC9	4.2	D7S 351	7cen-q22	
TN69	pBR322	2.6	D7S 352	7p?	
TN100L	Ch21A	1.8	D7S 353	7cen-q22	
TN100U	pBR322	2.5	D7S 354	7cen-q22	
TN112	pBR322	3.6	D7S 356	7cen-q22	
TN113	Ch21A	6.0	D7S 357		
TN119	pUC9	4.3	D7S 358		
TN122	pBR322	4.0	D7S 143		+
TN126	Ch21A	2.1	D7S 359		
TN127	Ch21A	3.3	D7S 144	7q32-q34	+
TN132	Ch21A	4.5	D7S 360	7q22	
TN143L	Ch21A	1.4	D7S 361		
TN143U	Ch21A	2.0	D7S 362		
TN156	Ch21A	1.7	D7S 363		
TN157	Ch21A	4.4	D7S 145	7p?	+
TN162	Ch21A	2.4	D7S 364	7cen-q22	
TN174U	Ch21A	3.5	D7S 146		+
TN177	Ch21A	4.8	D7S 147	7p	+
TN197	Ch21A	2.8			
TN198	Ch21A	2.8	D7S 148	7q31-q32	
TN200	Ch21A	1.9	D7S 365	7p14-cen	
TS74	pSV2-gpt	3.8	D7S 366	7cen-q22	
TS93	pUC9	3.0	D7S 149	7pter-p14	+
TS133	pSV2-gpt	2.3	D7S 367	7cen-q22	
TS134	Ch21A	1			
TS150	Ch21A	2.0			
TS194	pSV2-gpt	3.0	D7S 150	7p	+

[a] *Eco*RI fragments.

[b] As assigned by the Ninth Human Gene Mapping Workshop, Paris, September 1987.

[c] Regional assignments based on hybridization analysis with the cell hybrids and patient cell lines shown in fig. 1 (those with question marks are uncertain).

[d] Markers with detected RFLPs are indicated by a plus sign (+) (details in table 3).

respective RFLPs and *CF*. However, no recombination was detected between *CF* and eight of the DNA segments, *D7S391* (pB47L), *D7S118* (pB130), *D7S119* (pB174), *D7S120* (pB192), *D7S122* (pH131), *D7S113* (pJB5-21), *D7S126* (SC33), and *D7S340* (TM183) (table 3). The marker *D7S122* was particularly interesting, as it appeared to be closer to *CF* than did *MET* or *D7S8*. No recombination was detected between

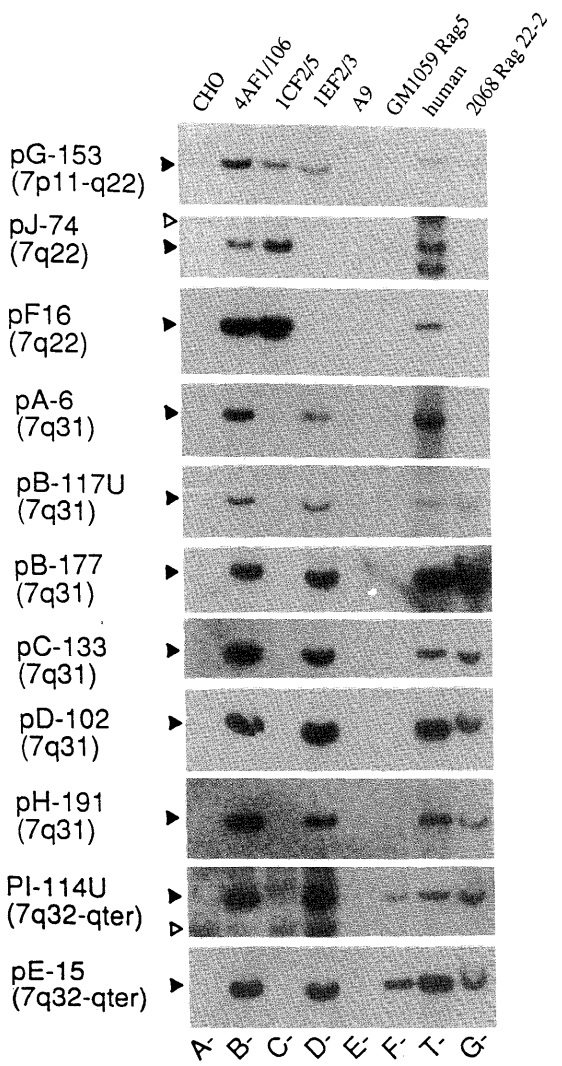

Figure 2 Examples of DNA marker hybridization analysis with DNA from the human-rodent somatic cell hybrids. DNA samples from the cell hybrids were digested with *Eco*RI, fractionated by electrophoresis in 0.7% agarose gels, transferred to Zetabind® membranes, and hybridized to [^{32}P]-labeled probe with standard procedures (see Material and Methods). The autoradiographs show the hybridization results with each of the indicated probes with DNA from CHO (lane A), 4AF1/104/KO15 (lane B), 1CF2/5/KO16 (lane C); 1EF2/3 (lane D); mouse A9 (lane E); GM1059Rag5 (lane F); human lymphoblasts (lane T), and 2068Rag22-2 (lane G). Signals indicate presence of homologous or related DNA sequences in the corresponding cell lines (solid triangles represent human-specific bands, and open triangles represent cross-hybridizing rodent sequences).

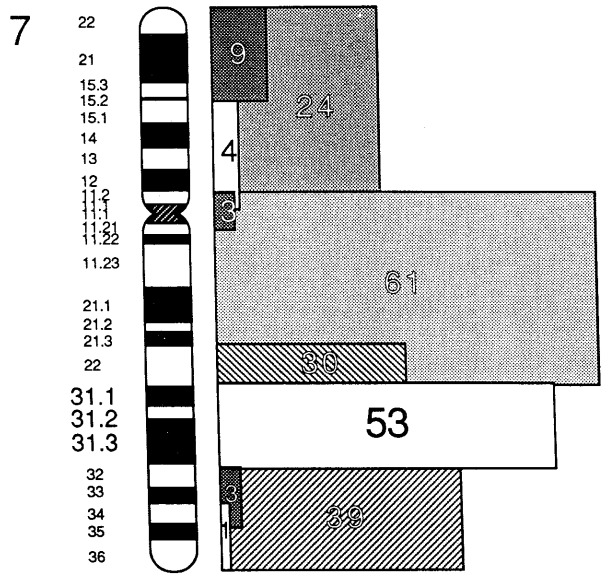

Figure 3 Regional localization of 258 chromosome 7 markers.

D7S122 and *CF* in 43 families with 228 informative meioses. The maximal lod ($\hat{z}$) score was 29.1 at θ = 0 with ($\hat{z}$-1) confidence limits of 0.00–0.01. Furthermore, *D7S122* did not appear to recombine with *CF* in two families that showed recombination between *CF* and *MET* or *D7S8* (fig. 4). These results suggest that *D7S122* is most likely to be between *MET* and *D7S8*, but the present data do not establish whether it lies on the centromeric or telomeric side of *CF*. The linkage relationships between *CF* and the other seven probes were not as conclusive because they were not informative in these two recombinant families.

Strong linkage disequilibrium was also detected between *D7S122* and *CF*. From the analysis of 176 unrelated parental chromosomes, the distribution of the large and small alleles of the *Hin*fI RFLP was 74 and 14 in the normal chromosomes, respectively, and it was 37 and 51 in the CF chromosomes. The correlation (Δ) value (Chakravarti et al. 1984) was .44, and the χ^2 was 32.3 with 1 df. In the same population, the Δ values were .32 (χ^2 = 17.1) for *CF-MET* metD *Ban*I) and .21 (χ^2 = 7.5) for *CF-D7S8* (pJ311 *Msp*I). The higher Δ value for *D7S122-CF* is thus consistent with the family data, which suggested that *D7S122* is closer to *CF* than is *MET* or *D7S8*.

In addition to the genetic analyses, a long-range mapping study has been conducted to investigate the physical relationships among all the DNA markers mapped to 7q31-q32. For each probe, the fragment sizes generated by 10 "rare-cutter" restriction enzymes were examined by pulsed-field gel electrophoresis and compared

Table 3

List of RFLPs detected with 43 Chromosome 7–specific DNA Probes

Probe	Region	Enzyme	Alleles	Lengths (kb)	Frequency	PIC
pA-21	7q32-qter	*Pst*I	1	4.4	.12	.19
			2	2.0	.88	
pA-37	7q31-q32	*Pst*I	1	20	.40	.36
			2	4.0	.60	
pA-51	7q31-q32	*Taq*I	1	3.8	.90	.16
			2	3.0	.10	
pB-47L	7q31-q32	*Bgl*II	1	2.2	.02	.04
			2	2.0	.98	
			3	1.6	<.01	
pB-48	7q31-q32	*Sst*I	1	7.0	.37	.36
			2	5.5	.63	
pB-130	7q31-q32	*Sau*3A	1	1.8	.53	.37
			2	1.6	.47	
		*Taq*I	1	7.2	.02	.04
			2	2.8	.98	
		*Nco*I	1	7.5	.55	.37
			2	5.5	.45	
pB-174	7q31-q32	*Taq*I	1	6.5	.08	.14
			2	4.5	.92	
		*Msp*I	1	6.7	.30	.33
			2	6.6	.70	
		*Stu*I	1	25	.50	.38
			2	20	.50	
pB-189	7q31-q32	*Bcl*I	1	6.2	.23	.29
			2	5.1	.77	
pB-192	7q31-q32	*Taq*I	1	9.0	.94	.11
			2	6.7	.06	
pC-67.1	7q31-q32	*Stu*I	1	3.3	.03	.04
			2	1.9 + 1.1	.97	
pC-102	7q31-q32	*Hae*III	1	1.8	.30	.33
			2	1.2	.70	
pE-79	7q32-qter	*Taq*I	1	2.2	.30	.33
			2	1.5	.70	
pF-167.2	7p31-q32	*Bgl*II	1	9.4	.93	.15
			2	8.0	.07	
pG-54	7q32-qter	*Pst*I	1	4.3	.63	.36
			2	1.5	.37	
pH-131	7q31-q32	*Hin*fI	1	.4	.65	.35
			2	.3	.35	
		*Dra*I	1	3.0	.69	.34
			2	1.8	.31	
pI-65	7q31-q32	*Ban*I	1	.5	.10	.16
			2	.4	.90	
pJ-18	7q31-q32	*Eco*RV	1	2.2	.39	.37
			2	1.9	.61	
pJB5-21	7q31-q32	*Sca*I	1	2.3	.08	.13
			2	1.3 + .9	.92	
		*Sph*I	1	3.1	.63	.35
			2	2.5	.37	
pJB89	7q31-q32	*Msp*I	1	12	.83	.25
			2	4.0	.17	
SA37	7q31-q32	*Pst*I	1	7.0	.23	.29
			2	5.0	.77	

Table 3 (continued)

Probe	Region	Enzyme	Alleles	Lengths (kb)	Frequency	PIC
SC33	7q31-q32	*Eco*RI	1	4.5	.18	.36
			2	4.0	.07	
			3	3.5	.75	
		*Hin*dIII	1	6.0	.18	.36
			2	5.5	.07	
			3	5.0	.75	
		*Bgl*II	1	3.2	.18	.36
			2	2.7	.07	
			3	2.2	.75	
SF32L	7q31-q32	*Alu*I	1	.7	.40	.36
			2	.4	.60	
SI32.....	7q31-q32	*Msp*I	1	2.3	.78	.36
			2	.5	.12	
			3	.4	.10	
		*Dra*I	1	1.5	.35	.35
			2	1.0 + .5	.65	
TD52	7pter-p14	*Pst*I	1	4.0	.85	.22
			2	1.0	.15	
TG16	7cen-q22	*Eco*RI	1	2.2	.70	.33
			2	1.0	.30	
		*Pvu*I	1	18	.10	.16
			2	12	.90	
TG105	7q32-q34	*Bgl*II	1	11.5	.36	.35
			2	10	.64	
		*Eco*RI	1	5.0	.11	.18
			2	3.3	.89	
TM60	7pter-p14	*Taq*I	1	5.3	.22	.28
			2	5.0	.78	
TM77	7pter-p14	*Taq*I	1	4.4	.40	.36
			2	2.1	.60	
TM102L	7pter-p14	*Taq*I	1	12	.57	.37
			2	8.5	.43	
TM138L	7	*Taq*I	1	9	.50	.38
			2	6	.50	
TM178	7	*Pst*I	1	2.4	.5	.38
			2	2.3	.5	
TM183	7q31-q32	*Taq*I	1	6.7	.02	.25
			2	6.0	.13	
			3	4.5	.85	
TM196	7q32-qter	*Pst*I		Many		
TN11	7	*Taq*I	1	8.0	.08	.14
			2	4.4	.92	
TN56	7	*Bgl*II	1	3.0	.67	.34
			2	2.4	.33	
TN66	7pter-p14	*Hin*dIII	1	20	.90	.16
			2	15	.10	
TN122	7	*Bgl*II	1	12	.30	.33
			2	7.0	.70	
		*Taq*I	1	9.0	.90	.16
			2	7.5	.10	
TN127	7q32-q34	*Taq*I	1	5.2	.76	.30
			2	4.4	.24	
TN157	7p	*Pst*I	1	5.0	.67	.34
			2	3.0	.33	

Table 3 (continued)

Probe	Region	Enzyme	Alleles	Lengths (kb)	Frequency	PIC
TN174U	7	*Taq*I		Many		
TN177	7p	*Taq*I	1	6.0	.32	.34
			2	3.3+3.0	.68	
		*Sst*I	1	4.6	.79	.28
			2	4.4	.21	
		*Pvu*II	1	5.5+1.7	.87	.20
			2	2.0	.13	
		*Pst*I		Many		
TS93	7pter-p14	*Pst*I	1	4.4	.32	.34
			2	4.2	.68	
TS194	7p	*Taq*I	1	1.5	.72	.32
			2	1.0	.28	

against each other. The result revealed that, while most of the markers yielded unique combinations of restriction-fragment sizes, *D7S122* (pH131) and *D7S340* (TM58) shared many common bands (fig. 5), suggesting that they are physically close to each other. In addition, pulsed-field gel mapping results indicated that both *D7S122* and *D7S340* are between *MET* and *D7S8* and that their order is *MET-D7S340-D7S122-D7S8* (data to be published elsewhere). *D7S122* and *D7S340* can therefore serve as new starting points in the search for the CF gene. However, since no recombination has been detected between *D7S122/D7S340* and *CF*, their relative order could not be established. The present study also has not allowed us to determine the relative position of *D7S122/D7S340* with respect to *D7S23*, the marker previously shown to be closely linked to *CF* (Estivill et al. 1987).

Discussion

The use of molecular genetic techniques, together with classical linkage analysis, has resulted in the

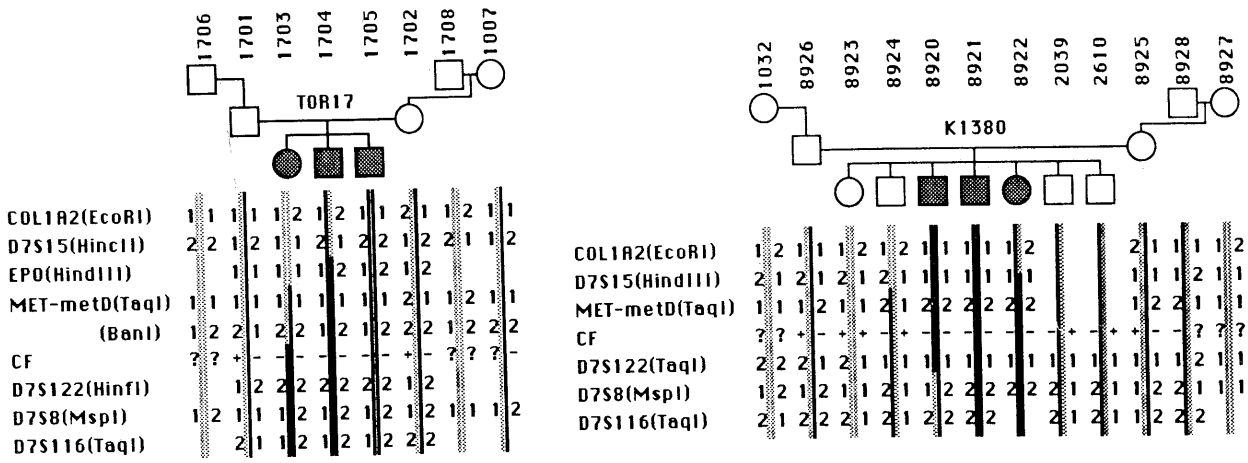

Figure 4 RFLP analysis of two families in whom recombination occurred between *CF* and *MET* (TOR17) or between *CF* and *D7S8* (K1380). The pedigrees are shown on the top, and the predicted chromosome haplotypes for each individual are shown on the bottom. The larger RFLP alleles for each of the indicated markers are denoted as allele 1. The putative, parental CF and the normal chromosomes are represented by solid and hatched lines, respectively. The markers, from top to bottom, are aligned according to genetic and physical mapping data presented here and elsewhere (see text), except that the relative order for *CF* and *D7S122* is not known.

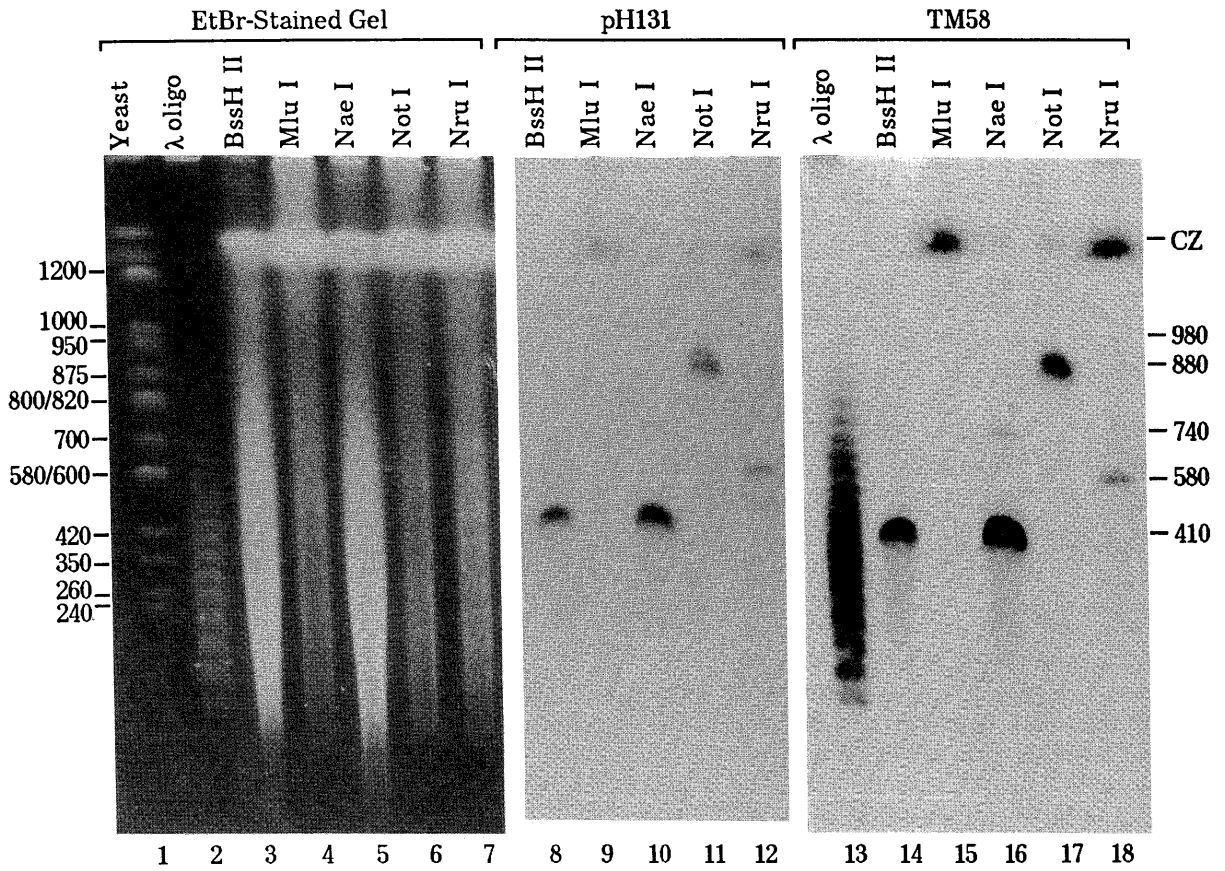

Figure 5 Pulsed-field gel analysis with pH131 (*D7S122*), and TM58 (*D7S340*). The DNA samples were prepared as described in Material and Methods and separated by electrophoresis in a crossfield gel apparatus with 45 mM Tris-borate/EDTA buffer (Maniatis et al. 1982) at 7.5V/cm for 24 h and a ramped switching time of 45–90 s. Lane 1, *Saccharomyces cereviseae* chromosomes as molecular weight markers; lanes 2 and 8, ladders of oligomerized bacteriophage λ DNA (48.5 kb); lanes 3, 8, and 14, lymphoblast DNA digested with *Bss*HII; lanes 4, 9, and 15, with *Mlu*I; lanes 5, 10, and 16, with *Nae*I; lanes 6, 11, and 17, with *Not*I; lanes 7, 12, and 18, with *Nru*I. The left-hand panel (lanes 1–7) shows the ethidium bromide–stained pattern of the gel; the middle panel (lanes 8–12) shows the blot hybridized with [^{32}P]-labeled pH131; and the right-hand panel (lanes 13–18) shows the blot hybridized with [^{32}P]-labeled TM58. Numbers on the sides are DNA fragment sizes in kilobases; the 980- and 740-kb bands are products of partial digestions with *Bss*HII and *Nae*I, respectively; CZ = compression zone.

identification of genetic loci and genes that are responsible for a variety of diseases with ill-defined biochemical causes (Davies et al. 1983; Gusella et al. 1983; Reeders et al. 1985; Tsui et al. 1985; Barker et al. 1987*b*; Bodmer et al. 1987; Simpson et al. 1987; Egeland et al. 1987; Leppert et al. 1987; Mathew et al. 1987; Rouleau et al. 1987; St. George-Hyslop et al. 1987; Seizinger et al. 1987). All of these discoveries were facilitated by the availability of DNA markers in the vicinity of the disease loci, and, in most cases, were due to the presence of RFLPs that could be followed in family studies. Patient samples with chromosome rearrangements have also served a major role in identifying the genes responsible for a number of disorders, namely Duchenne muscular dystrophy, chronic granulomatous disease, and retinoblastoma (Francke et al. 1985; Ray et al. 1985; Friend et al. 1986; Kunkel et al. 1986; Monaco et al. 1986; Royer-Pokora et al. 1986). Since no such material has been available from CF patients, this alternative is not applicable to CF research.

Estivill et al. (1987) were able to isolate a candidate gene from the *CF* region by using chromosome-mediated gene transfer (Scambler et al. 1986*a*) together with cloning procedures that selected for undermethylated CpG-rich DNA sequences. Their method exploited the cellular transforming properties of the activated met oncogene, but it might not be applicable to the study of other diseases if no selectable marker is located close

to the disease locus. The present study illustrates a general strategy of using a flow-sorted chromosome-specific library, somatic cell hybrids, family linkage analysis, linkage disequilibrium calculations, and pulsed-field gel electrophoresis toward the cloning of a disease gene. Although the CF gene has yet to be identified, this "saturation mapping" approach has allowed us to arrive at a close distance to *CF*, and chromosome walking and jumping experiments are now realistic. Collins et al. (1987) have constructed a general human chromosome jumping library for this particular purpose. Other approaches to identify additional DNA segments in a 1–2 megabase region—approaches such as cloning from pulsed-field gels (Michiels et al. 1987) and the use of yeast-cloning vector (Burke et al. 1987)—are also feasible with the closely linked markers presently available.

An important application of DNA markers closely linked to *CF* is for genetic diagnosis based on linkage. The RFLPs associated with *MET*, *D7S8*, and *D7S23* have already been used in practice extensively. The inclusion of *D7S122/D7S340* should increase the general informativeness of CF diagnosis, although full utility of these markers awaits a careful analysis of their relative order with respect to *CF* and *D7S23*. The other RFLPs identified in the present study should provide additional markers for the analysis and the construction of a fine genetic map of this chromosome. To this end, Barker et al. (1987*a*) reported a genetic linkage map of 63 DNA markers on human chromosome 7. A separate report by O'Connell et al. (1987) described a similar map with 20 markers. Two of the RFLPs described here, *D7S125* (SA37) and *D7S126* (SC33), have been localized between D7S8 and *TCRB* in the latter study, and their order has been determined to be *D7S8-DS125-D7S127-TCRB*.

The availability of the large number of DNA markers on chromosome 7 also provides us with an opportunity to study other disease conditions associated with this chromosome. A strong correlation has been found between monosomy 7 and acute nonlymphocytic leukemia, especially in those patients who have undergone aggressive irradiation and/or chemotherapy for a previous, malignant disease (Benitez et al. 1986). A significant portion of the deletions and translocations have been found to occur in specific regions of this chromosome (Rowley 1985). The breakpoints in these and other chromosome aberrations seem to coincide with the reported fragile sites on this chromosome (de la Chapelle and Benger 1984), suggesting that the observed instabilities are intrinsic to the specific chromosomal regions. With the present collection of probes and the technique of pulsed-field gel electrophoresis, it may be possible to clone the DNA from some of the regions and to begin to understand the underlying mechanisms that are responsible for these rearrangements.

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