Am. J. Hum. Genet. 43:471–475, 1988

# Recombinations between IRP and Cystic Fibrosis

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## Summary

**A candidate gene for cystic fibrosis was recently isolated by selective cloning of *Hpa*II-tiny-fragment islands; it maps considerably closer to *CF* than does MET or D7S8 (pJ3.11), and DNA polymorphisms from this region are in marked disequilibrium with CF. cDNA cloning has shown that this protein has a growth factor–like structure and shows homology to the murine and human proto-oncogene int-1; it is designated IRP (int-1-related protein). DNA sequences from the IRP locus that recognize RFLPs are proving to be highly informative for prenatal diagnosis. We report five crossovers that have been identified which occur either within the IRP locus or between IRP and *CF*; these recombinants demonstrate that *CF* maps between the DNA markers D7S8 and KM.19.**

## Introduction

The first linkage between a polymorphic marker and the cystic fibrosis mutation (*CF*) was with the serum protein paraoxonase (Eiberg et al. 1985), followed by linkage to several DNA markers located on the long arm of chromosome 7 (Knowlton et al. 1985, Tsui et al. 1985; Wainwright et al. 1985; White et al. 1985). A collaborative study demonstrated that the two closest markers, *MET* and D7S8, flank *CF* and are each approximately 1 centimorgan (cM) from the mutation (Beaudet et al. 1986).

A coding gene mapping between *MET* and D7S8 has been isolated by selection for *Hpa*II-tiny-fragment (HTF) islands from human transgenomes in mouse/human hybrid cell lines containing this region of chromosome 7 (Estivill et al. 1987*a*). The gene codes for a protein that is related to the murine oncogene int-1 and to the *Drosophila* segment polarity gene *wingless* and has been given the name "int-1-related protein" (IRP; Wainwright et al. 1988).

Several DNA sequences have been subcloned from IRP and the surrounding genomic sequences, each of which recognizes frequent RFLPs in Caucasians. Alleles detected by three probes (KM.19, CS.7, and XV-2c) show linkage disequilibrium with *CF*; this suggests that the majority of chromosomes carrying the CF mutation are from a single mutational event (Estivill et al. 1987*b*). KM.19 has been physically mapped 30 kb upstream (5′) of the IRP mRNA; CS.7 includes most of the HTF island within which transcription starts; and XV-2c is a noncoding sequence in the middle of the gene, approximately 20 kb downstream from CS.7. Figure 1 summarizes the physical and genetic localizations for the markers D7S8, D7S23, *MET*, and D7S18 surrounding *CF*; physical localizations are based on pulsed-field gel-electrophoresis mapping experiments (Poustka et al., in press), and genetic locations are based on cystic fibrosis (Beaudet et al. 1986) and multipoint (Lathrop et al. 1988) mapping studies.

These markers are being extensively used for family studies for carrier testing and prenatal diagnosis of cystic

Received March 4, 1988; revision received May 2, 1988.

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0002-9297/88/4304-0014$02.00

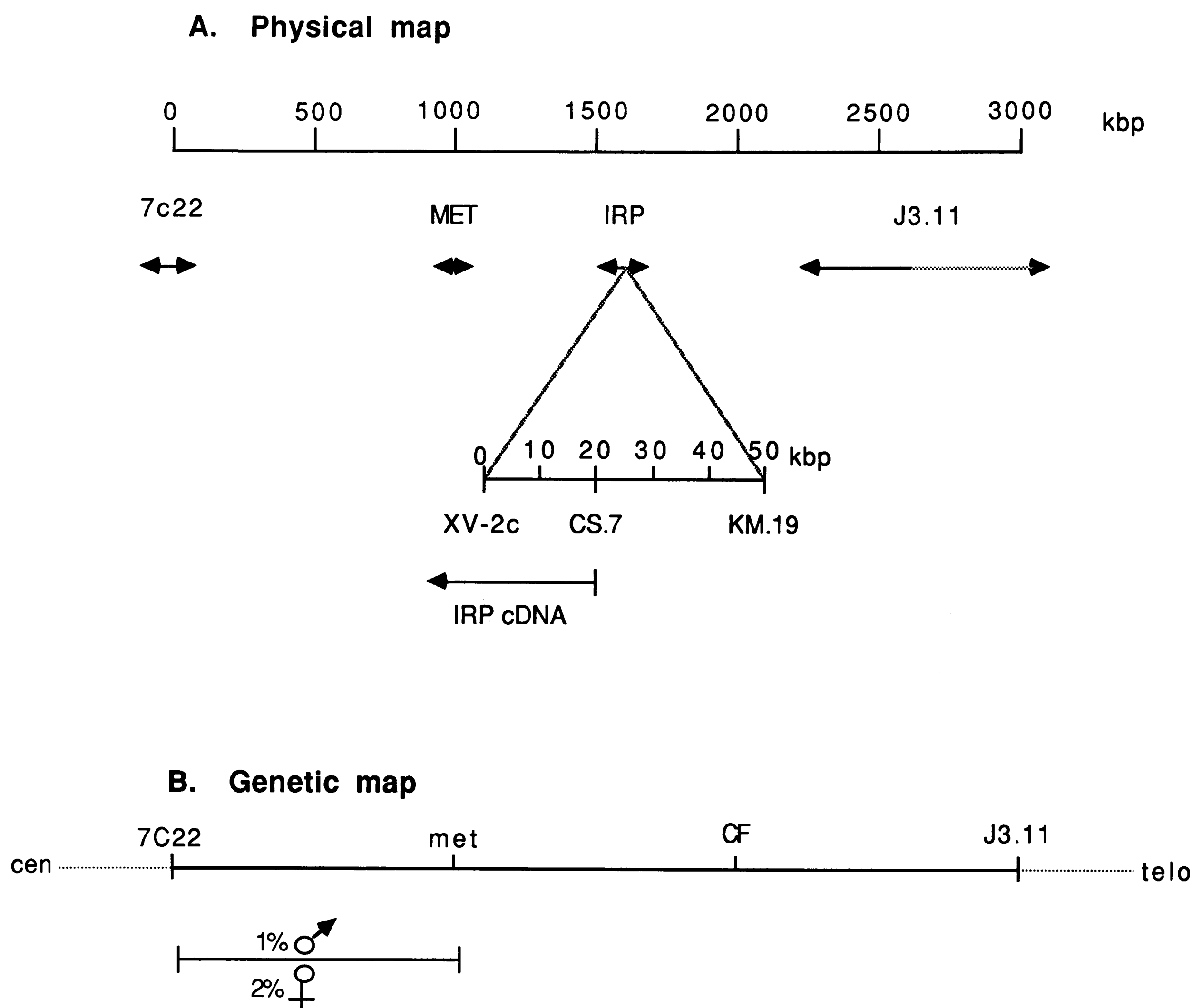


**Figure 1** Physical and genetic localization of three DNA probes closely linked to and flanking the cystic fibrosis locus

fibrosis (Beaudet et al. 1988; Estivill et al. 1988). We report here five families in which there is evidence of recombination either within the IRP locus or between IRP and cystic fibrosis. These data show that *CF* maps between D7S8 and KM.19.

## Material and Methods

### *Families*

Five nuclear CF families were ascertained, as they show recombination between CF and DNA probes that map within 1 cM. Diagnosis in each family was confirmed after measurement of elevated sweat electrolytes. Paternity was confirmed by multilocus haplotyping. Family 1 has been previously reported in detail by Berger et al. (1987). Families 4 and 5, each with one living affected child, were genotyped using DNA probes when they requested first-trimester prenatal diagnosis. When it was apparent that probe-probe recombination made accurate diagnosis impossible, the families elected to continue the pregnancies to the second trimester, at which time microvillar enzymes were estimated in amniotic fluid (Carbans et al. 1983; Boue et al. 1986). In each case, analysis of the enzyme levels predicted an affected fetus and both pregnancies were terminated and fetal products were collected.

### *DNA Probes*

The following probes were informative in this study: 7C22 (D7S18) (Scambler et al. 1986), metD (White et al. 1985), XV-2c and CS.7 (D7S23) (Estivill et al. 1987*a*), KM.19 (Estivill et al. 1987*b*), pJ3.11 (D7S8) (Cooper et al. 1985; Wainwright et al. 1985) and CRI-S14 (D7S73), CRI-S23 (D7S78), CRI-S94 (D7S87), and CRI-S140 (D7S93) (Barker et al. 1987). DNA preparation, endonuclease digestion, fractionation on agarose gels, transfer to membranes, and hybridization were all by standard methods.

## Results

Table 1 shows the DNA genotypings for the informative matings in each of the five families. Probes are tabulated for each family in centromere-to-telomere order. Examination of haplotypes shows a recombinant pattern in each case; families 1, 2, and 5 show evidence of recombination in female meiosis, and family 3 shows a recombination in male meiosis. Family 4 shows a double intercross, and recombination may not be assigned.

Family 5 is informative for probes flanking the crossover which is assigned to the 20-kb interval between XV-2c and CS.7.

**Table 1**

**Informative DNA Genotypings for Five Recombinant CF Families, with the National Origin of Each Family Shown in Parentheses**

A. Family 1 (German Democratic Republic)

| Probe/enzyme | Father | Mother | CF Child 1 | CF Child 2 | Normal Child |
|---|---|---|---|---|---|
| D7S18/*Eco*RI | 1–1 | 1–2 | 1–1 | 1–2 | 1–2 |
| metD/*Taq*I | 1–1 | 1–2 | 1–1 | 1–2 | 1–2 |
| XV-2c/*Taq*I | 1–1 | 1–2 | 1–2 | 1–1 | 1–1 |

B. Family 2 (France)

| Probe/enzyme | Father | Mother | CF Child 1 | CF Child 2 |
|---|---|---|---|---|
| XV-2c/*Taq*I | 1–1 | 1–2 | 1–2 | 1–1 |
| KM.19/*Pst*I | 2–2 | 1–2 | 1–2 | 2–2 |

C. Family 3 (Italy)

| Probe/enzyme | Father | Mother | CF Child 1 | CF Child 2 | CF Child 3 |
|---|---|---|---|---|---|
| metD/*Taq*I | 1–2 | 1–1 | 1–1 | 1–2 | 1–2 |
| XV-2c/*Taq*I | 1–2 | 2–2 | 2–2 | 1–2 | 1–2 |
| KM.19/*Pst*I | 1–2 | 1–1 | 1–1 | 1–2 | 1–2 |

D. Family 4 (Yugoslavia)

| Probe/Enzyme | Father | Mother | CF Child 1 | CF Fetus 1 |
|---|---|---|---|---|
| metD/*Ban*I | 1–2 | 1–2 | 1–2 | 1–1 |
| KM.19/*Pst*I | 1–2 | 1–2 | 1–2 | 2–2 |
| D7S8/*Msp*I | 1–2 | 1–2 | 2–2 | 2–2 |

E. Family 5 (United States)

| Probe/Enzyme | Father | Mother | CF Child 1 | CF Fetus 1 |
|---|---|---|---|---|
| D7S78/*Taq*I | 1–2 | 1–2 | 1–2 | 2–2 |
| D7S73/*Msp*I | 1–1 | 1–2 | 1–1 | 1–2 |
| XV-2c/*Taq*I | 1–1 | 1–2 | 1–1 | 1–2 |
| CS.7/*Hha*I | 1–2 | 1–2 | 2–2 | 2–2 |
| KM.19/*Pst*I | 1–2 | 1–2 | 2–2 | 2–2 |
| D7S8/*Msp*I | 1–1 | 1–2 | 1–1 | 1–1 |
| D7S87/*Taq*I | 1–2 | 1–2 | 1–1 | 1–1 |
| D7S93/*Msp*I | 1–2 | 1–2 | 2–2 | 2–2 |

## Discussion

There is very strong evidence to show that *CF* maps genetically between *MET* and D7S8 (Beaudet et al. 1986; Lathrop et al. 1988). If this gene order is accepted, analysis of haplotypes in these five families shows that *CF* is excluded from the interval KM.19-*MET* and must lie between D7S8 and KM.19. The consistent and strong disequilibrium between KM.19/CS.7 and *CF* (Estivill et al. 1987*a*, 1987*b*) argues that *CF* is likely to map within several tens of kilobases of KM.19.

Although we have not requested groups using probes to tell us the number of nonrecombinant families tested with the closest probes, we believe this to be of the order of several thousand. This gives a recombination fraction of the order of 0.1 cM, which is consistent with our estimate of the genetic distance on the basis of the disequilibrium data (Estivil et al. 1987*a*, 1987*b*).

Two genetic mapping studies of chromosome 7q have concluded that there is a sex difference in recombination of approximately 2:1, female:male (Barker et al. 1987; Lathrop et al. 1988). In this study there are 3 female:1 male recombinants (one recombinant being unassigned), a finding in broad agreement with previous data. This sex difference should be considered when calculating genetic risks for families seeking prenatal diagnosis or carrier detection/exclusion. This is particularly pertinent when diagnosis is dependant on the inheritance of a chromosome tracked by MET, D7S8, or D7S18, markers that show at least 1% recombination with *CF*. Errors due to recombination are insignificant when diagnosis is based on flanking markers; however, these counseling situations are infrequent, as J3.11 is the sole freely available polymorphic marker sufficiently tightly linked on the telomeric flank of *CF* and is only moderately informative.

The recombinant families reported here collectively exclude IRP as the CF gene. IRP is most probably a secreted "growth factor" molecule and is an unlikely candidate for the CF gene (Wainwright et al., 1988), as the protein that is defective in CF is likely to be a membrane-associated or intracellular regulator of chloride permeability (Li et al. 1988).

## Acknowledgments

This work was funded by the U.K. Cystic Fibrosis Research Trust and the U.K. Medical Research Council, the Howard Hughes Medical Institute and Cystic Fibrosis Foundation (USA), the French Cystic Fibrosis Foundation (A.F.L.M.), and the Progetto Finalizzato "Ingegneria Genetica e Basi Molecolari delle Malattie Ereditarie," CNR, Italy. We thank our clinical colleagues and the CF families for their continued and unstinting support.

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