Am J Hum Genet 39:694–698, 1986

Further Linkage Data on Cystic Fibrosis: The Utah Study

RAY WHITE,[1,2] MARK LEPPERT,[1] PETER O'CONNELL,[1] YUSUKE NAKAMURA,[1] SCOTT WOODWARD,[1] MARK HOFF,[1] JOHN HERBST,[3] MICHAEL DEAN,[4] GEORGE VANDE WOUDE,[4] G. MARK LATHROP,[1] AND JEAN-MARC LALOUEL[1,2]

SUMMARY

We reported earlier complete linkage between cystic fibrosis and an RFLP of the *met* proto-oncogene revealed by the probe p*met*H. Another clone, p*met*D, detects another polymorphism with the *Taq*I restriction enzyme. Further linkage studies, now involving 22 families, have confirmed the tight linkage of cystic fibrosis to the MET and D7S8 loci. Significant allelic association was found between CF and allelic series defined by the p*met*H probe.

INTRODUCTION

Recent linkage findings in cystic fibrosis (CF) are summarized in the accompanying joint paper [1]. We had already reported [2] very tight linkage between CF and a genetic marker defined by a cloned DNA fragment of the *met* proto-oncogene, p*met*H [3, 4]. Twelve of the 13 families tested were informative for linkage; a maximum lod score of 8.65 was obtained at a recombination value of zero, the confidence upper-bound on the latter estimate being .05. We have since identified another clone at the MET locus, p*met*D, which detects a poly-

Received July 24, 1986.

M. D. is an American Cancer Society postdoctoral fellow. R. J. and J.-M. L. are investigators of the Howard Hughes Medical Institute.

[1] Howard Hughes Medical Institute and the Cystic Fibrosis Foundation Research Center, University of Utah Medical Center, Salt Lake City, UT 84132.

[2] Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT 84132.

[3] Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT 84132.

[4] Litton Bionetics, Inc., Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701.

morphism with the *Taq*I restriction enzyme. We now report linkage data obtained on an extended sample set with several DNA clones: p*met*H and p*met*D at the MET locus; pJ3.11 [5], defining the D7S8 locus; phage 6, defining the D7S11 locus [6]; and COL1A2 [7]. Loose linkage between CF and the latter marker had been reported earlier [5, 8].

MATERIALS AND METHODS

An enlarged panel of 22 families, each having at least two sibs affected with CF, was constituted through the Utah Cystic Fibrosis Research Center. Our earlier report on linkage between CF and p*met*H in 13 families [2] documented the methods used in preparing the samples and performing the Southern experiments. In the extended studies reported here, we included a new clone, p*met*D, a 1.1-kilobase (kb) *Eco*RI restriction fragment of the *met* proto-oncogene previously described [3]. It detects polymorphic *Taq*I fragments at approximately 6.0 kb and 4.4 kb, with frequencies of .78 and .22, respectively (fig. 1). The samples were also screened with two other DNA clones assigned to chromosome 7: COL1A2 and the arbitrary DNA sequence phage 6 (D7S11). Data for the COL1A2 polymorphisms were collected with the restriction enzymes *Eco*RI and *Msp*I; those for phage 6 were collected with *Hin*dIII.

Linkage analysis was performed with the LINKAGE programs [9, 10]. Haplotypes were constructed by studying the distribution of alleles in affected siblings and their parents in families without recombination events. Allelic associations were investigated by standard log-linear models [11].

RESULTS

The probe p*met*H detects a diallelic polymorphism with both the *Taq*I and *Msp*I restriction enzymes. However, these two allelic series are in strong linkage disequilibrium, revealing a third haplotype only in the family (1402/1407) segregating a rare, 5.0-kb *Msp*I allele detected with the probe p*met*H. However, the diallelic system revealed by p*met*D with the enzyme *Taq*I shows

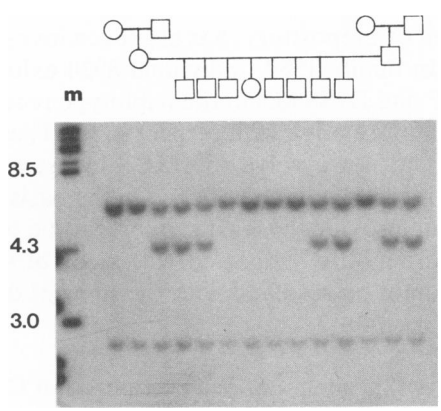

FIG. 1.—Segregation of the *Taq*I polymorphism detected with p*met*D. Autoradiogram of a DNA blot of *Taq*I-digested DNA samples of individuals in a multigeneration Utah pedigree (*above*), hybridized with ^{32}P-labeled p*met*D. Molecular weight standards (*m*) are indicated in kilobases.

TABLE 1

PAIRWISE LOD SCORES IN 22 CF FAMILIES

Loci	Recombination	Confidence limits	Lod score
CF .... MET	.010	.00–.05	16.82
CF D7S8	.014	.00–.07	10.85
CF COLIA2	.500	.12–.50	0.00
CF D7S11	.500	.15–.50	0.00
MET .. D7S8	.012	.00–.05	15.41

much less linkage disequilibrium and therefore contributes additional information. These three polymorphisms were combined into haplotypes to generate a single allelic series. The same operation was performed to combine the two polymorphic systems revealed by the enzymes *Taq*I and *Msp*I with the probe pJ3.11 into a single allelic series at the D7S8 locus. A similar operation was carried out with the *Eco*RI and *Msp*I data for the COL1A2 locus.

Linkage Analysis

After construction of haplotypes, four loci were investigated in our families: MET, D7S8, COL1A2, and D7S11. The results extended the observation of tight linkage between the CF, MET, and D7S8 loci but detected no support for tight linkage between the CF, COL1A2, and D7S11 loci. Pairwise lod scores, recombination estimates, and their one-lod-unit confidence limits are reported in table 1. Only one recombinant was observed for each pair of loci, CF-MET and CF-D7S8. The families in which these events occurred are documented in figure 2. In family 1076, a recombination event between the CF and MET loci must have occurred from a haplotype received from the father in one of the two affected offspring tested. Unfortunately, the information available at the D7S8 locus does not allow us to address the issue of gene order. This family, which belongs to the Camden cell repository, has also been investigated by others [1], with similar results. In family 1380, individual 8920 exhibits a recombination event between the CF and D7S8 loci in the haplotype received from his father. This individual is also informative with respect to MET, and it appears that the recombination event did not involve the MET locus, a result confirmed by resampling and retesting all family members. On the basis of this unique observation, the present data provide the least support for the order CF-D7S8-MET. The two alternative orders are supported with odds of 61:1 against the least favored order, but cannot be resolved with the present data.

Allelic Associations

In our earlier report [2], allelic association between CF and MET reached marginal significance. Our enlarged dataset now yields significant evidence of allelic association between CF and the two allelic series p*met*H-*Taq*I (χ^2 = 9.27) and p*met*H-*Msp*I (χ^2 = 8.71), which are themselves in very strong linkage disequilibrium; other tests of association, particularly with the locus

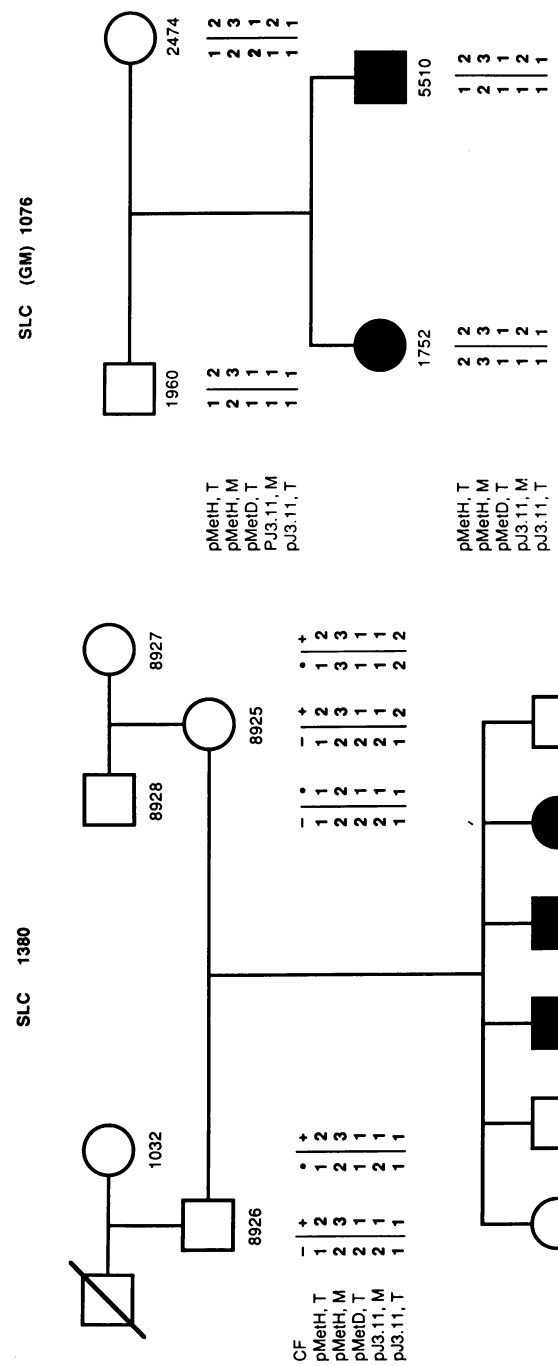

FIG. 2.—Segregation data for families recombinant between CF and linked DNA markers. Recombinant families are shown with phenotype and DNA marker allele distribution per chromosome indicated where known. Affected individuals are shown with *solid circles* or *squares*. In family SLC 1380, individual 8920 has inherited a haplotype recombinant (*line*) between CF and D7S8 from his father (8926). In family SLC (GM) 1076, a recombinant haplotype between CF and MET from father (1960) has been inherited by one of the two affected children.

D7S8, were not significant. Data and results are presented in the accompanying joint report [1].

CONCLUSION

Tight linkage between CF and both the MET and D7S8 loci supports the notion that the vast majority of cystic fibrosis cases result from mutation at a single locus. Whether a variety of molecular defects at this locus can lead to cystic fibrosis must await the characterization of the CF gene. The available genetic markers are already of value for medical diagnosis, however, and they will be of major significance in progress toward eventual identification of the CF gene.

ACKNOWLEDGMENTS

We thank F. Ramirez for clones defining the COL1A2 locus, J. Schmidtke for the J3.11 clone, and A. Bowcock for phage 6. We also gratefully acknowledge Barbara Ogden for sample collection; Tara Bragg, John Cowan, Russell Eldridge, Mary Hadley, Leslie Jerominski, Lesa Nelson, and Leslie Sargent for technical assistance; Carol Wood of the Intermountain Cystic Fibrosis Center for assistance in family collection; the NIGMS Human Genetic Mutant Cell Repository for lymphoblastoid cell lines of family 774; and Ruth Foltz for editorial assistance in preparing this manuscript.

REFERENCES

1. Beaudet A, Bowcock A, Buchwald M, et al.: Linkage of cystic fibrosis to two tightly linked DNA markers: joint report from a collaborative study. 39:681–693, 1986
2. White R, Woodward S, Leppert M, et al.: A closely linked genetic marker for cystic fibrosis. *Nature* 318:382–384, 1985
3. Cooper CS, Park M, Blair DG, et al.: Molecular cloning of a new transforming gene from a chemically transformed human cell line. *Nature* 311:29–33, 1984
4. Dean M, Park M, Le Beau MM, et al.: The human *met* oncogene is related to the tyrosine kinase oncogenes. *Nature* 318:385–388, 1985
5. Wainwright BJ, Scambler PJ, Schmidtke J, et al.: Localization of cystic fibrosis locus to human chromosome 7cen-q22. *Nature* 318:384–385, 1985
6. Feder J, Yen L, Wijsman E, et al.: A systematic approach for detecting high-frequency restriction fragment length polymorphisms using large genomic probes. *Am J Hum Genet* 37:635–649, 1985
7. Tsipouras P, Myers JC, Ramirez F, Prockop D: RFLP associated with the pro-alpha2(I) gene of human type I procollagen. *J Clin Invest* 72:1262–1267, 1983
8. Tsui L-C, Buchwald M, Barker D, et al.: Cystic fibrosis locus defined by a genetically linked polymorphic DNA marker. *Science* 230:1054–1057, 1985
9. Lathrop GM, Lalouel J-M: Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 36:460–465, 1984
10. Lathrop GM, Lalouel J-M, Julier C, Ott J: Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 81:3443–3446, 1984
11. Bishop YMM, Fienberg SE, Holland PW: *Discrete Multivariate Analysis: Theory and Practice*. Cambridge, Mass., MIT Press, 1975