Am J Hum Genet 39:681–693, 1986

Linkage of Cystic Fibrosis to Two Tightly Linked DNA Markers: Joint Report from A Collaborative Study

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SUMMARY

A collaborative study involving seven research groups provided an opportunity to investigate the linkage relationships between cystic fibrosis and two DNA marker loci, MET and pJ3.11 (D7S8), on an extended sample of 211 tested families. The maximum lod scores, recombination estimates, and confidence upper bounds (in parentheses) were 91.0 at $\theta = .004$ (.012) for CF and MET, 71.3 at $\theta = .003$ (.011) for CF and D7S8, and 69.3 at $\theta = .018$ (.036) for MET and D7S8. Three-locus analyses yielded best support for the order MET-CF-D7S8, with odds against the alternate orders CF-MET-D7S8 and CF-D7S8-MET of 9:1 and 161:1, respectively. However, the number of observed recombinants was small and only one of the recombinants was jointly informative for all three markers. Significant allelic association was found between CF and both MET and D7S8. Weaker association between the latter two loci is consistent with the order MET-CF-D7S8.

Received July 24, 1986.

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INTRODUCTION

Since the initial report of linkage between cystic fibrosis (CF) and a polymorphism of the enzyme paraoxonase (PON) [1, 2], further findings have emerged at a rapid pace. The discovery of linkage between CF and the arbitrary DNA probe DOCR1-917 (D7S15) with a recombination rate of .15 [3], and subsequent localization of D7S15 to chromosome 7 [4], was followed by the identification of two closely linked DNA markers; p*met*H, a DNA segment of the *met* proto-oncogene (MET) [5], and pJ3.11 [D7S8), an arbitrary cloned sequence [6]. No recombination events involving the latter two markers were observed with CF in either initial report. Another clone, p*met*D, detects further polymorphism at the MET locus [7]. Because close linkage has implications for medical diagnosis as well as strategic relevance for the characterization of the gene involved in CF, these tightly linked probes were made available to the scientific community. As a result, seven groups agreed to share their data for a joint analysis. Additional markers, including the pro-alpha2(I) collagen gene (COL1A2) and the T-cell receptor beta gene (TRCB), were also found to be linked, although less tightly, to CF [8, 9], and these two markers also were tested in several of the collaborating laboratories.

MATERIALS AND METHODS

Family Data

Data were contributed by seven centers, identified by the following abbreviations: CAL (Stanford and Berkeley), FRA (Framingham), HOU (Houston), LON (London), SAN (San Antonio), SLC (Salt Lake City), and TOR (Toronto). A total of 211 families, each with at least two affected members, were used for the joint analyses. A number of families were tested by more than one group. Consequently, we deleted duplicate families for which genotypic data on MET and D7S8 were not yet complete, including: TOR 6, SLC 1076 and TOR 17 (GM 1076), SAN 1038 (GM 1038), SAN 1080 (GM 1080), TOR 10 (GM 1078), and LON 22 to LON 33 (Danish families). An unusual family (TOR 19) with two affected sibs discordant for both alleles at the D7S8 locus, discussed in [10], was also eliminated from the joint analysis. Additional information for the study of allelic association was provided by the London group.

DNA preparation and Southern experiments were performed following protocols described in the accompanying reports contributed by each research group.

Linkage analyses were carried out with the LINKAGE programs [11, 12]. Heterogeneity of CF was evaluated by the method described in [13]. Whenever feasible, haplotypes were determined for the study of allelic associations by scoring CF and normal chromosomes in the parents of affected children; only one parental pair was scored for extended pedigrees with multiple affected individuals. Contingency-table analysis was used to measure the statistical significance of linkage disequilibrium, as well as heterogeneity between laboratories. For loci with two frequent alleles, the strength of the association was measured using the correlation: $c = (n_{ii}n_{jj} - n_{ij}n_{ji})/[(n_{i.}n_{j.}n_{.i}n_{.j})\ 0.5]$, where n_{ij} is the observed number of haplotypes consisting of allele i at the first locus and allele j at the second locus, and $n_{i.}$ and $n_{.j}$ are, respectively, the observed frequencies of allele i at the first locus, and allele j at the second locus. Relative risk was calculated as: $r = (n_{ii} + 0.5)(n_{jj} + 0.5)/[(n_{ij} + 0.5)(n_{ji} + 0.5)]$.

RESULTS

Linkage Analysis

Summary statistics on the familial data available for linkage analysis are presented in table 1. Pairwise linkage results are summarized in table 2. Consistent with previous reports, MET and D7S8 are tightly linked to CF. The recombination estimates, together with their confidence upper bound of one lod unit [14], are .004 (.012) for CF and MET and .003 (.011) for CF and D7S8. The recombination estimate for MET and D7S8 is .018, with a one-unit confidence region of .008–.036. Families exhibiting recombinants for any pair of these three loci are presented in table 3. Two recombinants were observed between CF and MET, one between CF and D7S8, and six between MET and D7S8. The estimate of heterogeneity for CF, that is, the proportion of unlinked families, is estimated to be zero, and the 95% confidence interval allows up to 5% heterogeneity.

In order to infer the linear order of these loci, three-point tests were performed (table 4). The best supported order is MET-CF-D7S8, although the odds favoring this order against the alternative CF-MET-D7S8 are only 9:1. The order CF-D7S8-MET is 161-fold less likely than the best supported order. Only one recombinant family (SLC 1380) is informative for both MET and D7S8; individual 8920 (see fig. 2) in [7]) shows recombination between CF and D7S8 and between MET and D7S8, but none between CF and MET. Hence, all

TABLE 1

SUMMARY OF FAMILIAL DATA: NO. AFFECTED AND UNAFFECTED INDIVIDUALS TYPED FOR EACH OF 15 ALLELIC SYSTEMS

Allelic system		Affected	Normal	Total
p*met*H,	*Taq*I	420	787	1207
p*met*H,	*Msp*I	183	313	496
p*met*D,	*Taq*I	374	690	1064
p*met*D,	*Ban*I	31	40	71
pJ3.11,	*Msp*I	427	796	1223
pJ3.11,	*Taq*I	247	419	666
pJ3.11,	*Stu*I ...	35	44	79
PON	.....	200	308	508
DOCR1-917,	*Hinc*II ..	156	173	329
DOCR1-917,	*Hin*dIII ..	121	162	283
COL1A2,	*Rsa*I	135	159	294
COL1A2,	*Eco*RI ...	211	373	584
COL1A2,	*Msp*I	180	294	474
TCRB,	*Bgl*II	164	228	392
DOCR1-917,	*Hinc*II ..	156	173	329
DOCR1-917,	*Hin*dIII ..	121	162	283
Phage 6,	*Hin*dIII ..	84	117	201

TABLE 2

PAIRWISE LINKAGE TESTS

Loci		Recombination	Confidence limits	Lod score
CF	MET	.004	.00–.01	91.01
CF	D7S8	.003	.00–.01	71.28
CF	PON	.140	.08–.23	4.26
CF	D7S15	.170	.11–.26	4.25
CF	COL1A2 ..	.172	.12–.25	6.12
CF	TCRB	.186	.11–.33	2.37
MET	D7S8	.018	.01–.04	69.31
MET	PON	.218	.13–.34	2.70
MET	D7S15	.224	.14–.35	2.35
MET	COL1A2 ..	.211	.15–.30	4.98
MET	TCRB	.230	.12–.44	1.09
D7S8	PON	.259	.15–.41	1.57
D7S8	D7S15	.232	.14–.37	1.95
D7S8	COL1A2 ..	.190	.12–.27	5.38
D7S8	TCRB	.174	.10–.31	2.62
PON	D7S15	.046	.01–.13	7.89
PON	COL1A2 ..	.056	.01–.16	5.35
PON	TCRB	.471	.22–.50	0.01
D7S15	COL1A2 ..	.062	.03–.13	11.12
D7S15	TCRB	.342	.18–.50	0.20
COL1A2	TCRB	.186	.11–.33	2.37

the evidence against the order CF-D7S8-MET is based on this single observation.

Allelic Associations

Association between CF and marker alleles was first evaluated separately for p*met*H, p*met*D, and pJ3.11. As shown in table 5, all allelic systems, except pJ3.11-*Taq*I, are in significant linkage disequilibrium with CF in one or more laboratories. Overall, the correlations with CF are similar for the MET allelic

TABLE 3

FAMILIES EXHIBITING RECOMBINATION EVENTS

Family (individual)		CF-MET	CF-D7S8	MET-D7S8
HOU 1076	(1752)	R	.	.
LON 38	(3 or 5)	R	.	.
SLC 1380	(8920)	NR	R	R
CAL 41	(195)	.	.	R
LON 11	(3)	.	.	R
LON 13	(4 and 7)	.	.	R
LON 21	(3)	.	.	R
SAN 7	(3, 4 and 6) or (7 and 8)	.	.	R

NOTE: R = recombination events; NR = no recombination events; . = no information.

TABLE 4

TESTS OF GENE ORDERS

Locus order	Recombination estimates		Odds
MET-CF-D7S8	.001	.006	1
CF-MET-D7S8	.003	.015	9
CF-D7S8-MET	.002	.015	161

NOTE: The odds reported are against a given order vs. the best supported order, MET-CF-D7S8.

systems and pJ3.11-*Msp*I. However, disequilibrium with p*met*H-*Taq*I is significant only in the data from Salt Lake City and Toronto. This may be due to significant heterogeneity among laboratory samples in the allelic frequencies for normal chromosomes.

Disequilibrium varies significantly between different laboratories for p*met*D (table 6). Comparison of the gene frequencies in different laboratories reveals significant heterogeneity for the p*met*D alleles on CF chromosomes. This is due to an elevated frequency of the 4.4-kilobase (kb) allele (allele 2) in the data contributed by London; no further evidence of heterogeneity is found after the exclusion of the London data ($\chi^2 = 8.52$ with 5 d.f.; $.75 < P < .9$).

Linkage disequilibrium between the marker loci was evaluated statistically for the allelic systems defined by p*met*H-*Taq*I, p*met*D-*Taq*I, and pJ3.11-*Msp*I (table 7). The p*met*H-*Taq*I and p*met*H-*Msp*I systems are in nearly complete disequilibrium (data not shown), and as the latter was not typed in all laboratories, it was not included in the haplotype analysis. The pJ3.11-*Taq*I system was also excluded because it shows little polymorphism and was not typed in all laboratories.

There is no detectable association between the MET and D7S8 loci on normal chromosomes (table 8). However, on chromosomes carrying the CF allele, the MET-D7S8 association becomes significant at $P = .05$ (table 7) if all independent haplotypes from all relatives in extended families, and in recombinant families that can be scored unambiguously, are included. Limiting the analysis to nuclear families and nonrecombinant chromosomes accounts for the slight differences reflected in table 8 as compared to table 7. Within the MET locus, p*met*H and p*met*D are in strong linkage disequilibrium. One rare p*met*H-p*met*D haplotype was found, only in the LON and FRA data sets; there is still evidence of heterogeneity in the frequencies for both CF ($\chi^2 = 24.42$; 12 d.f.; $P < .025$) and normal chromosomes ($\chi^2 = 24.72$; 12 d.f.; $P < .025$) if this haplotype is removed. Three-locus haplotype data presented in table 9 show that the strongest association, as measured by relative risk, occurs with the haplotype defined by the presence of the 7.5-kb p*met*H-*Taq*I, 6.2-kb p*met*D-*Taq*I, and 4.2-kb pJ3.11-*Msp*I fragments. There is significant heterogeneity between laboratories regarding the three-locus haplotype frequencies for normal chromosomes ($\chi^2 = 49.54$; 30 d.f.; $P < .025$) but not for CF chromosomes ($\chi^2 = 41.92$;

TABLE 5

DISTRIBUTION OF THE TWO MOST FREQUENT ALLELES FOR THE MET AND D7S8 LOCI

LOCUS AND ALLELES	LABORATORY	ALLELES: CF 1	CF 2	NORMAL 1	NORMAL 2	RISK	CHI-SQUARE	CORRELATION
p*met*H *Taq*I	CAL	26	18	28	15	0.78	0.34	−.06
Alleles:	FRA	43	21	39	18	0.95	0.02	−.01
1 = 7.5 kb	HOU	31	19	32	17	0.87	0.12	−.03
2 = 4.0 kb	LON	89	46	84	43	0.99	0.00	.00
	SAN	15	9	15	9	1.00	0.00	.00
	SLC	29	11	15	24	4.05	9.27	.34
	TOR	65	26	49	42	2.12	6.01	.18
Total		298	150	262	168	1.27	2.97	.06
p*met*H *Msp*I	HOU	30	18	32	17	0.89	0.08	−.03
Alleles:	LON	7	1	3	3	5.00	2.36	.41
1 = 2.3 kb	SAN	16	6	13	8	1.60	0.57	.12
2 = 1.8 kb	SLC	29	11	14	22	3.98	8.71	.34
	TOR	25	7	19	11	2.00	1.64	.16
Total		107	43	81	61	1.86	6.50	.15
p*met*D *Taq*I	CAL	37	1	29	7	6.36	5.41	.27
Alleles:	FRA	62	2	41	13	8.13	11.58	.31
1 = 6.0 kb	HOU	43	7	38	11	1.73	1.19	.11
2 = 4.4 kb	LON	83	23	81	23	1.02	0.01	.01
	SAN	19	3	13	8	3.51	3.38	.28
	SLC	34	6	32	6	1.06	0.00	.01
	TOR	77	5	62	20	4.62	10.61	.25
Total		355	47	296	88	2.23	17.40	.15
pJ3.11 *Msp*I	CAL	34	22	26	27	1.59	1.50	.12
Alleles:	FRA	34	24	21	29	1.93	2.97	.17
1 = 4.2 kb	HOU	26	24	14	35	2.65	5.64	.24
2 = 1.8 kb	LON	67	59	56	69	1.40	1.76	.08
	SAN	12	12	8	16	1.94	1.37	.17
	SLC	23	17	21	17	1.09	0.04	.02
	TOR	55	39	32	58	2.53	9.72	.23
Total		251	197	178	251	1.79	18.53	.15
pJ3.11 *Taq*I	CAL	25	1	23	2	1.80	0.40	−.03
Alleles:	HOU	47	3	47	2	0.71	0.19	−.04
1 = 6.3 kb	SAN	23	1	22	2	1.74	0.36	.09
2 = 3.1 kb	SLC	38	2	37	1	0.62	0.30	−.06
	TOR	57	9	63	3	0.33	3.30	−.16
Total		190	16	192	10	1.33	0.63	−.06

TABLE 6

CHI-SQUARE VALUES FOR HETEROGENEITY TESTS OF GENE FREQUENCIES AND LINKAGE DISEQUILIBRIUM OF MET AND D7S8 WITH THE CF GENE

TEST	SYSTEM				
	pmetH *TaqI*	pmetH *MspI*	pmetD *TaqI*	pJ3.11 *MspI*	pJ3.11 *TaqI*
Gene frequency differences by laboratory:					
CF chromosomes	3.39 (6)	3.62 (4)	21.10***(6)	2.03 (6)	4.55 (4)
Normal chromosomes	13.49*(6)	6.99 (4)	4.06 (6)	10.24 (6)	1.54 (4)
Disequilibrium with CF†	4.54 (1)	8.57 (1)	16.74***(1)	18.80***(1)	1.38 (1)
Variation of disequilibrium by laboratory	13.10*(6)	6.97 (4)	16.90** (6)	4.57 (6)	3.56 (4)

NOTE: * $.05 \geq P > .01$, ** $.01 \geq P > .005$, *** $.005 \geq P$. Degrees of freedom are given between the parentheses following the chi-square statistic. The test statistics are nonsignificant unless otherwise indicated.

† Taking account of possible laboratory heterogeneity of the marginal gene frequencies at each locus.

30 d.f.; $.10 < P < .05$). Third-order linkage disequilibrium between CF, MET, and D7S8 is not significant (table 8).

Carrier Status of Unaffected Siblings

For the 228 unaffected siblings of CF children, carrier status based on MET and D7S8 was assessed. Frequencies of carrier, noncarrier, and partially informative siblings are shown in table 10. If the partially informative siblings are equally distributed, as expected, between carriers and noncarriers, the proportion of noncarriers would be (57 + 25)/228 = .360 and of carriers (121 + 25)/228 = .640. This difference is not significant ($\chi^2 = 0.97$); indeed, this test lacks power, for it would require 25,836 observations to detect at the 5% level a differential fitness accounting for an equilibrium frequency of 1/40 for the CF gene [15].

DISCUSSION

Based on the available data from 211 families with at least two affected individuals, upper bounds of the recombination estimates between CF and MET or D7S8 are .012 and .011, respectively. These markers are likely to be flanking the locus responsible for CF. The finding of significant linkage disequilibrium is consistent with the inference of very tight linkage; by reference to disequilibrium values observed in the HLA region [16], the genomic segment spanned by these three loci may be of the order of 2,000–3,000 kb.

It should be noted, however, that the inference of gene order relies on very few, rare recombination events. While all recombinant individuals have been confirmed by resampling and retesting, the frequency of these events may be approaching the range of expected laboratory error. Future observations of recombination events in this region may lead to a revision of this inference. Because families in which recombinants were identified were not fully informa-

TABLE 7

TWO-LOCUS HAPLOTYPES FOR CF AND NORMAL CHROMOSOMES WITH pmetH (*TaqI*), pmetD (*TaqI*), AND pJ3.11 (*MspI*)

LOCI	LAB	CHROMOSOME	ALLELES 11	12	21	22	χ^2	CORRELATION
pmetH pJ3.11	CAL	CF	17	6	10	7	1.01	.15
		Normal	13	12	7	4	0.58	−.13
	FRA	CF	18	16	11	3	2.72	−.24
		Normal	9	21	9	6	3.75	−.28
	HOU	CF	15	15	9	7	0.16	−.05
		Normal	8	20	3	12	0.38	.09
	LON	CF	28	34	17	8	3.72	−.21
		Normal	22	35	18	14	2.58	−.17
	SAN	CF	7	7	3	5	0.32	.12
		Normal	5	8	2	6	0.40	.14
	SLC	CF	17	11	6	4	0.00	.01
		Normal	8	5	12	11	0.29	.09
	TOR	CF	35	20	9	11	2.10	.17
		Normal	12	22	7	24	1.27	.14
	Total	CF	137	109	65	45	0.36	−.03
		Normal	77	124	58	77	0.73	−.05
pmetD pJ3.11	CAL	CF	24	10	1	0	0.41	−.11
		Normal	14	11	5	2	0.54	−.13
	FRA	CF	37	28	1	1	1.66	.15
		Normal	20	30	5	10	0.08	.03
	HOU	CF	25	19	1	5	3.41	.26
		Normal	10	25	2	8	0.29	.08

	LON	CF	46	28	7	11	3.21	.19
		Normal	33	42	9	8	0.44	−.07
	SAN	CF	8	11	1	2	0.08	.06
		Normal	5	10	3	4	0.19	.09
*pmet*D pJ3.11	SLC	CF	12	17	5	4	0.00	.01
		Normal	25	21	5	3	0.18	.06
	TOR	CF	40	32	2	2	0.05	.02
		Normal	14	35	3	10	0.13	.04
	Total	CF	202	145	18	25	4.16	.10
		Normal	121	174	32	45	0.01	−0.01
*pmet*H *pmet*D	CAL	CF	20	1	11	0	0.54	−.13
		Normal	12	7	12	0	8.11	−.43
	FRA	CF	38	2	21	1	0.01	−.01
		Normal	24	12	17	0	7.32	−.37
	HOU	CF	25	7	18	0	4.58	−.30
		Normal	21	11	17	0	7.54	−.39
	LON	CF	45	11	6	0	1.43	−.15
		Normal	43	10	9	4	0.88	.12
	SAN	CF	11	3	8	0	1.98	−.30
		Normal	7	7	7	0	5.25	−.50
	SLC	CF	23	6	11	0	2.68	−.26
		Normal	9	5	23	0	9.50	−.51
	TOR	CF	53	5	23	0	2.11	−.16
		Normal	24	17	36	0	19.15	−.50
	Total	CF	215	35	98	1	12.93	−.19
		Normal	140	69	121	4	40.70	−.35

NOTE: Alleles are defined in table 5.

TABLE 8

CHI-SQUARE VALUES FOR TESTS OF LINKAGE DISEQUILIBRIUM BETWEEN THE MET AND D7S8 LOCI

TEST	HAPLOTYPES		
	pmetH pJ3.11 *TaqI MspI*	pmetD pJ3.11 *TaqI MspI*	met* pJ3.11 *MspI*
Normal chromosomes:			
(a) Disequilibrium	0.61 (1)	0.09 (1)	0.30 (2)
(b) Variation by laboratory	8.33 (6)	2.19 (6)	6.65 (12)
CF chromosomes:			
(a) Disequilibrium	0.31 (1)	2.95 (1)	3.76 (2)
(b) Variation by laboratory	9.94 (6)	4.95 (6)	15.15 (12)
Third-order linkage disequilibrium	0.01 (1)	1.69 (1)	4.17 (2)

NOTE: Degrees of freedom are given between parentheses following the chi-square statistics. All tests are nonsignificant.

* pmetH *TaqI* and pmetD *TaqI* haplotype.

tive with respect to both marker loci, more definitive inference of gene order could be provided by the discovery of further polymorphisms at these loci.

The present data do not provide evidence of heterogeneity with respect to a presumed CF gene, but the apparent absence of genetic heterogeneity does not preclude the existence of distinct molecular defects leading to disease at this locus. Linkage disequilibrium of MET and D7S8 appears to be higher for CF chromosomes than for normal chromosomes. If confirmed by further data, this result may indicate that independent mutations to cystic fibrosis were at most few in number, or linkage disequilibrium would have been destroyed.

The data presented here have immediate implications for prenatal diagnosis and genetic counseling for CF. With the present estimates of confidence upper bounds for recombination between CF and either MET or D7S8, the accuracy of diagnosis in a fully informative family is 96%. This emphasizes the importance of obtaining DNA data on patients seriously ill with CF and of offering DNA analysis and genetic counseling to a family when the index case is diagnosed. The presence of linkage disequilibrium can significantly affect the risk for a random individual to be a carrier of the CF gene, as a function of his haplotypic composition (table 9): thus, a homozygote for the haplotype (1,1,1) has approximately a 25-fold greater risk of being a carrier than a homozygote for the haplotype (2,1,2).

The availability of flanking DNA markers would significantly improve the accuracy of diagnosis using molecular probes. While MET and D7S8 are likely to be flanking the CF locus, confirmation must await the identification of further polymorphism at these loci in order to make the rare recombination events observed fully informative for linkage. Increased polymorphism at these loci will also raise the proportion of families in which genotypic diagnosis can be proposed.

TABLE 9

DISTRIBUTION OF p*met*H (*Taq*I), p*met*D (*Taq*I), AND pJ3.11 (*Msp*I) HAPLOTYPES FOR CF AND NORMAL CHROMOSOMES

	HAPLOTYPE					
p*met*H	1	2	1	1	1	2
p*met*D	1	1	1	2	2	1
LABORATORY pJ3.11	1	1	2	1	2	2
CAL:						
CF chromosomes	13	8	5	1	0	3
Normal chromosomes	7	5	3	5	2	3
Relative risk	1.8	1.5	1.6	0.3	0.2	...
FRA:						
CF chromosomes	15	14	16	1	1	3
Normal chromosomes	5	8	24	3	6	7
Relative risk	6.0	1.7	2.4	0.9	0.5	...
HOU:						
CF chromosomes	14	9	8	1	5	9
Normal chromosomes	4	4	12	2	8	11
Relative risk	3.9	2.5	0.8	0.7	0.8	...
LON:						
CF chromosomes	17	14	17	5	9	3
Normal chromosomes	13	8	19	5	4	12
Relative risk	4.6	6.1	3.2	3.6	7.5	...
SAN:						
CF chromosomes	4	3	6	1	1	5
Normal chromosomes	2	2	4	3	3	3
Relative risk	1.1	0.9	0.9	0.3	0.3	...
SLC:						
CF chromosomes	12	6	10	4	2	4
Normal chromosomes	6	12	3	3	2	12
Relative risk	5.3	1.4	8.3	3.5	2.8	...
TOR:*						
CF chromosomes	29	8	18	2	2	10
Normal chromosomes	5	7	9	2	9	22
Relative risk	11.5	2.4	4.2	2.1	0.6	...
Total:						
CF chromosomes	104	62	80	15	20	37
Normal chromosomes	42	46	64	23	34	70
Relative risk	4.6	2.5	2.3	1.2	1.1	...

NOTE: Alleles are defined in table 5. Haplotypes are given in increasing order of risk in the total sample. Risks have been calculated relative to the haplotype 2, 1, 2.

* Typings were completed in some families after this analysis was finished. Review counts are given Tsui et al. (this issue).

NOTE ADDED IN PROOF: Family TOR 19, in which three out of the four chromosomes in the offspring appeared to involve a recombination event between CF and MET and D7S8, was initially considered questionable, and therefore was not included in the analysis reported above. Moreover, significant heterogeneity was indicated when this family was included (see Tsui et al., this issue). When the linkage analyses were repeated with TOR 19 included, the following results were obtained: (1) the maximum lod scores, recombination estimates,

TABLE 10

CARRIER STATUS OF UNAFFECTED SIBLINGS OF CF PATIENTS

Sample	Carriers	Noncarriers	Indeterminate	Total
CAL	18	3	6	27
FRA	8	3	5	16
HOU	12	8	4	24
LON	6	6	6	18
SAN	37	14	12	63
SLC	24	8	8	40
TOR	16	15	9	40

and confidence upper bounds were 89.3 at $\theta = .005$ (.015) for CF and MET, 67.8 at $\theta = .007$ (.018) for D7S8, and unchanged for MET and D7S8; (2) the order CF-MET-D7S8 was better supported than MET-CF-D7S8 with odds of 5:1, a result not readily reconciled with the disequilibrium data. New polymorphisms identified at this locus will help resolve the issue.

ACKNOWLEDGMENTS

The present collaboration was initiated at a meeting in Toronto (December 16–17, 1985), sponsored by the Cystic Fibrosis Foundation and hosted by Manuel Buchwald and Lap-Chee Tsui. The authors wish to thank Tami Elsner for her technical assistance in the joint analyses and Ruth Foltz for editorial assistance in the preparation of this manuscript. The authors would also like to acknowledge the following support: The Cystic Fibrosis Foundation and the Howard Hughes Medical Institute (A. B., J.-M. L., and R. W.); National Institutes of Health (N.I.H.) grants AM-34942, GM-28428, and CA-27632 (A. B., L. C.-S., and M.-C. K.); the Canadian Cystic Fibrosis Foundation, N.I.H., the North Dakota Cystic Fibrosis Foundation, and the Sellers Fund from the Hospital for Sick Children, Toronto (M. B. and L.-C. T.); the Cystic Fibrosis Research Trust and the Medical Research Council, U.K. (M. F., B. W., and R. W.); N.I.H. grants HL-31916, AM-34948, P30-AM 27651, and AM-34917 (K. K. and P. W.); N.I.H. grant AM34992 and March of Dimes grant 1-927 (S. N.). The participation of clinicians and of CF families is deeply appreciated.

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