

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.

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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; *pmetH*, 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, *pmetD*, 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: $c = (n_{ij}n_{ji} - n_{ij}n_{ji}) / [(n_i n_j n_i n_j) 0.5]$, 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 |
|-------------------------------------|----------|--------|-------|
| <i>pmetH</i> , <i>TaqI</i> | 420 | 787 | 1207 |
| <i>pmetH</i> , <i>MspI</i> | 183 | 313 | 496 |
| <i>pmetD</i> , <i>TaqI</i> | 374 | 690 | 1064 |
| <i>pmetD</i> , <i>BanI</i> | 31 | 40 | 71 |
| <i>pJ3.11</i> , <i>MspI</i> | 427 | 796 | 1223 |
| <i>pJ3.11</i> , <i>TaqI</i> | 247 | 419 | 666 |
| <i>pJ3.11</i> , <i>StuI</i> | 35 | 44 | 79 |
| PON | 200 | 308 | 508 |
| DOCR1-917, <i>HincII</i> .. | 156 | 173 | 329 |
| DOCR1-917, <i>HindIII</i> .. | 121 | 162 | 283 |
| COL1A2, <i>RsaI</i> | 135 | 159 | 294 |
| COL1A2, <i>EcoRI</i> | 211 | 373 | 584 |
| COL1A2, <i>MspI</i> | 180 | 294 | 474 |
| TCRB, <i>BglII</i> | 164 | 228 | 392 |
| DOCR1-917, <i>HincII</i> .. | 156 | 173 | 329 |
| DOCR1-917, <i>HindIII</i> .. | 121 | 162 | 283 |
| Phage 6, <i>HindIII</i> .. | 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 *pmetH*, *pmetD*, and *pJ3.11*. As shown in table 5, all allelic systems, except *pJ3.11-TaqI*, 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-*MspI*. However, disequilibrium with *pmetH-TaqI* 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 *pmetD* (table 6). Comparison of the gene frequencies in different laboratories reveals significant heterogeneity for the *pmetD* 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 *pmetH-TaqI*, *pmetD-TaqI*, and pJ3.11-*MspI* (table 7). The *pmetH-TaqI* and *pmetH-MspI* 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-*TaqI* 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, *pmetH* and *pmetD* are in strong linkage disequilibrium. One rare *pmetH-pmetD* 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 *pmetH-TaqI*, 6.2-kb *pmetD-TaqI*, and 4.2-kb pJ3.11-*MspI* 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 ($\chi^2 = 41.92$;

| LOCUS AND ALLELES | LABORATORY | CF | | NORMAL | | Risk | CHI-SQUARE | CORRELATION |
|--|------------|---------|-----|--------|------|-------|------------|-------------|
| | | 1 | 2 | 1 | 2 | | | |
| | | ALLELES | | | | | | |
| <i>pmeH TaqI</i> Alleles: 1 = 7.5 kb 2 = 4.0 kb | CAL | 26 | 18 | 28 | 15 | 0.78 | 0.34 | .06 |
| | FRA | 43 | 21 | 39 | 18 | 0.95 | 0.02 | -.01 |
| | HOU | 31 | 19 | 32 | 17 | 0.87 | 0.12 | -.03 |
| | 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 |
| <i>pmerH MspI</i> Alleles: 1 = 2.3 kb 2 = 1.8 kb | HOU | 30 | 18 | 32 | 17 | 0.89 | 0.08 | -.03 |
| | LON | 7 | 1 | 5 | 3 | 5.00 | 2.36 | .41 |
| | SAN | 16 | 6 | 13 | 8 | 1.60 | 0.57 | .12 |
| | 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 |
| <i>pmerD TaqI</i> Alleles: 1 = 6.0 kb 2 = 4.4 kb | CAL | 37 | 1 | 29 | 7 | 6.36 | 5.41 | .27 |
| | FRA | 62 | 2 | 41 | 13 | 8.13 | 11.58 | .31 |
| | HOU | 43 | 7 | 38 | 11 | 1.73 | 1.19 | .11 |
| | 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 |
| <i>pJ3.11 MspI</i> Alleles: 1 = 4.2 kb 2 = 1.8 kb | CAL | 34 | 22 | 26 | 27 | 1.59 | 1.50 | .12 |
| | FRA | 34 | 24 | 21 | 29 | 1.93 | 2.97 | .17 |
| | HOU | 26 | 24 | 14 | 35 | 2.65 | 5.64 | .24 |
| | 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 |
| <i>pJ3.11 TaqI</i> Alleles: 1 = 6.3 kb 2 = 3.1 kb | CAL | 25 | 1 | 23 | 2 | 1.80 | 0.40 | -.03 |
| | HOU | 47 | 3 | 47 | 2 | 0.71 | 0.19 | -.04 |
| | SAN | 23 | 1 | 22 | 2 | 1.74 | 0.36 | .09 |
| | 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 |

TABLE 6

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

| TEST | SYSTEM | | | | |
|---|-----------------------------|-----------------------------|-----------------------------|------------------------------|------------------------------|
| | <i>pmetH</i> <i>TaqI</i> | <i>pmetH</i> <i>MspI</i> | <i>pmetD</i> <i>TaqI</i> | <i>pJ3.11</i> <i>MspI</i> | <i>pJ3.11</i> <i>TaqI</i> |
| 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 | | | | | | χ^2 | CORRELATION |
|---------------------|--------------|--------------|---------|----|----|------|------|------|----------|-------------|
| | | | 11 | 12 | 21 | 22 | 22 | 22 | | |
| <i>pmetH</i> 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 | | | |
| <i>pmetD</i> 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 |
| 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 |
| 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 | | |
|--|---|---|-----------------------------------|
| | <i>pmetH</i> pJ3.11 <i>TaqI MspI</i> | <i>pmetD</i> pJ3.11 <i>TaqI MspI</i> | <i>met*</i> pJ3.11 <i>MspI</i> |
| 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 *pmetH* (*TaqI*), *pmetD* (*TaqI*), AND *pJ3.11* (*MspI*) HAPLOTYPES FOR CF AND NORMAL CHROMOSOMES

| LABORATORY | HAPLOTYPE | | | | | | |
|------------|--------------------------|------|-----|-----|-----|-----|-----|
| | <i>pmetH</i> | 1 | 2 | 1 | 1 | 1 | 2 |
| | <i>pmetD</i> | 1 | 1 | 1 | 2 | 2 | 1 |
| | <i>pJ3.11</i> | 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.

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