Genetic homogeneity of cystic fibrosis

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ABSTRACT

We studied large Amish/Mennonite/Hutterite kindreds that segregate cystic fibrosis (CF) for linkage between CF and the polymorphic DNA markers pJ3.11 and 7C22 located on chromosome 7. These inbred pedigrees consist of more than 300 members including 30 affected individuals. In these families, linkage between the CF locus and the chromosome 21 marker D21S5 and between CF and the marker at the *met* oncogene locus on chromosome 7 had been previously indicated. We now report linkage between CF and pJ3.11 ($\chi^2=4.92$, $P=0$) and between CF and 7C22 ($\chi^2=3.42$, $P=0$). Therefore, CF segregates in these large pedigrees in a manner consistent with data from smaller outbred families with respect to the markers on chromosome 7 closest to CF. These data are consistent with locus homogeneity for the defect causing CF in the populations that have been examined to date.

INTRODUCTION

Cystic fibrosis (CF) is a common autosomal recessive disease with a carrier frequency of approximately 1:20 in Northern Europeans, for which the primary biochemical abnormality is unknown. The clinical features include pancreatic insufficiency, chronic lung infection in many cases, and characteristic elevated sweat electrolyte levels.

There have been extensive attempts to localize the CF gene by identification of linked polymorphic markers. The first genetic linkage was demonstrated between CF and the polymorphic enzyme marker paraoxinase (PON) [1]. This discovery was followed closely by the report of linkage between CF and the DNA marker DOCRI-917 (D7S15) [2] which was subsequently mapped to chromosome 7 [3]. Both of these markers are relatively distant from the CF locus. The maximum likelihood estimate of the recombination frequency is 0.15 between CF and PON and 0.17 between CF and D7S15 [4]. Three DNA markers closely linked to CF have recently been reported: *meth* [5, 6], pJ3.11 [7], and 7c22 [8]. The combined data from several groups suggests that these markers are likely to be within a few centimorgans of the CF locus, with *meth* and pJ3.11 within 1cm. [4].

We have previously described inbred communities that have a high incidence of CF, presumably by founder effect [9, 10]. At the 1985 American Society of Human Genetics meeting, we reported tentative linkage between CF and the chromosome 21 marker D21S5 in two large pedigrees from these inbred communities ($\hat{\theta}=0.06$, $\hat{z}=2.48$). We have subsequently extended the analysis of D21S5 and markers at the MET oncogene locus within these pedigrees. Tentative linkage to D21S5 remained, but close linkage was established between CF and MET [11]. We were unable to determine the reason for these apparently conflicting results although the possibility of locus heterogeneity could not be ruled out.

Large pedigrees are especially helpful in addressing the question of locus heterogeneity since highly significant lod scores can often be obtained from a single pedigree. Genetic heterogeneity is an important question because of the pressing need to use linked DNA markers for prenatal diagnosis, carrier detection, and as stepping stones for isolating the CF gene. Although the earlier finding in these families of linkage between the CF locus and markers at the met locus is consistent with a locus for CF on chromosome 7, the testing of additional linked markers is necessary in order to further resolve the question of locus heterogeneity.

Here we report significant linkage between CF and pJ3.11, and between CF and 7C22, in two large inbred pedigrees. These results strongly favor locus homogeneity for CF in the populations tested in that markers shown to be linked to CF in smaller outbred nuclear families are now shown to be linked to CF in these larger pedigrees.

MATERIALS AND METHODS

Pedigrees

Probands in inbred populations were identified and the pedigrees constructed as previously described [9, 11]. Two large extended kindreds with CF were studied, one (Amish-Mennonite, A-M) containing 21 affected individuals and the other (Hutterite, H) containing 9 affected individuals.

DNA Analysis

DNA was prepared either directly from peripheral blood lymphocytes or from Epstein-Barr virus-transformed lymphoblastoid cell lines. Lymphoblast DNA was prepared using methods previously described [12]. Five micrograms of DNA were digested overnight using the conditions suggested by the supplier, using 10 units of restriction enzyme. The resulting DNA fragments were separated on 0.8% agarose gels and transferred to a positively charged nylon

membrane (Zetabind, AMF Cuno) using a modified form of Southern blotting [13].

DNA probes were labeled by nick-translation [14] with ^{32}P -dCTP to specific activities of 2×10^8 cpm/ug. Unincorporated nucleotide was removed by centrifugation through small columns of Sepharose CL-4B. Filters were prehybridized for 24 hrs, washed in 3xSSC at 22°C for 4 hrs, and hybridized in 6xSSC, 0.3%SDS, 1xDenhardt's and 500 ug/ml denatured calf thymus DNA at 65°C for 24-72 hrs. Filters were then washed in successive changes of 2x and 1xSSC with a final wash in 0.1xSSC, 0.1% SDS at 65°C before autoradiography at -70°C for 1-5 days.

DNA Probes

pJ3.11 detects a diallelic MspI polymorphism with alleles of 4.2 and 1.8 kb and frequencies of 45% and 55%, respectively. 7C22 detects a diallelic EcoRI polymorphism with alleles of 7.2 and 5.1 kb with frequencies of 75% and 25%, respectively. Meth and metD, provided by Dr. R. White, are polymorphic markers at the met oncogene locus; each detects a Taq I polymorphism. For linkage analysis, we assumed no recombination between the meth and metD TaqI sites and no linkage disequilibrium and therefore treated the met polymorphisms as markers at a single locus forming four haplotypes at frequencies of 44%, 35%, 12%, and 9%. Allele frequencies were calculated from members of the populations under study and did not differ significantly from those previously reported.

Linkage Analysis

The calculations for two-point analyses were performed with the computer program LINKAGE v.3.4 [15] assuming no sex difference in recombination. Maximal likelihood estimates of the recombination fraction and lod score were calculated using the program VACO3 [16]. The confidence interval for the maximal likelihood estimate of the recombination fraction was determined by graphic interpolation [17].

RESULTS

The A-M and H pedigrees are composed of 28 nuclear families. 23 families were tested with the markers pJ3.11, 7C22, meth, and metD. Of these, 15 families were informative for pJ3.11, with at least one parent in each family heterozygous for the MspI polymorphism. 16 families were informative for the EcoRI polymorphism detected by 7C22. 18 families were informative for the met markers. A standard two-point analysis using the lod score method to detect linkage of these markers to CF and to each other was

Table 1. Linkage analysis of pJ3.11, 7C22, met, and CF.

	θ	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	$\hat{z}$	$\hat{\theta}$	confidence interval
CF--pJ3.11		4.92	4.23	3.55	2.89	2.24	1.65	1.13	0.68	0.33	4.92	0	0-0.07
CF--7C22		3.42	3.15	2.69	2.19	1.70	1.24	0.82	0.46	0.19	3.42	0	0-0.13
pJ3.11--7C22			7.28	6.72	5.99	5.15	4.19	3.29	2.30	1.33	7.32	0.03	0-0.13
pJ3.11-- <u>met</u>		11.89	10.72	9.49	8.23	6.93	5.60	4.25	2.91	1.64	11.89	0	0-0.04
7C22-- <u>met</u>		9.40	8.53	7.60	6.64	5.64	4.60	3.56	2.50	1.47	9.40	0	0-0.05

Lod scores were computed at conventional values of the recombination fraction (θ). The maximal likelihood estimates of the recombination fraction and lod score are $\hat{\theta}$ and $\hat{z}$, respectively.

performed (Table 1). The maximal likelihood estimates of the LOD score ($\hat{z}$) and the recombination fraction ($\hat{\theta}$), assuming equal male and female recombination, are $\hat{z}=4.92$, $\hat{\theta}=0$ between CF and pJ3.11; and $\hat{z}=3.42$, $\hat{\theta}=0$ between CF and 7C22. The confidence intervals are 0-0.07 and 0-0.13, respectively. Both markers are linked to CF with odds in favor of linkage of approximately 83,000:1 and 2,600:1, respectively. Linkage between pJ3.11 and 7C22 ($\hat{z}=7.32$, $\hat{\theta}=0.03$), pJ3.11 and met ($\hat{z}=11.89$, $\hat{\theta}=0$), and between 7C22 and met ($\hat{z}=9.40$, $\hat{\theta}=0$) is demonstrated. These results are consistent with a locus for CF on chromosome 7 in the members of these pedigrees.

Although there is no evidence for recombination between the markers and the CF locus, one recombinant was observed between the pJ3.11 and 7C22 loci. This crossover, however, occurred in an unaffected sibling of a family which did not segregate the markers at the met locus in an informative manner. Therefore, we were unable to infer by standard three point analysis a favored map order for the CF and marker loci.

DISCUSSION

CF has generally been held to be inherited as a single locus, autosomal recessive trait [18-26]. However, arguments have been made for multiple loci on the basis of gene frequency and/or clinical (phenotypic) heterogeneity. The reliability of following the inheritance of CF by the analysis of linked markers depends heavily on the absence of non-allelic (locus) heterogeneity. If genetic heterogeneity for the CF locus exists (i.e., if more than one locus were involved) it would be revealed by the demonstration of distinct linkage groups with CF in different sets of families. It is therefore important to determine whether heterogeneity does in fact exist by testing a large number of families with the currently available markers. Large pedigrees, such as those studied here, are especially well-suited for

providing answers to these types of questions. While we favor the single locus hypothesis, heterogeneity was a possible explanation of our earlier linkage data in the Amish-Mennonite and Hutterite pedigrees where linkage to a chromosome 21 marker (D21S5) was indicated. In these pedigrees, the apparent linkage between CF and D21S5 may be due to chance, to an interchange of DNA between chromosomes 7 and 21, or for some other reason which is not at present understood. However, the linkage of CF to met, pJ3.11, and 7C22 in these families strongly supports a locus for CF within the established chromosome 7 linkage group. Since the chromosome 7 markers have, so far, all demonstrated tight linkage to CF when tested against outbred nuclear families, the data presented in this paper is consistent with genetic homogeneity for the CF locus in all populations tested to date.

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