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Brief Communication

Homogeneity of Cystic Fibrosis in Italy

E. VITALE,[1] M. DEVOTO,[1] G. MASTELLA,[2] AND G. ROMEO[1]

SUMMARY

In 12 unrelated Italian cystic fibrosis (CF) families the frequencies of four DNA polymorphisms closely linked to the CF gene on chromosome 7 were quite similar to those reported for other population samples. Among the 23 affected children from the 12 families, only one recombinant occurred between the CF gene and the *met* locus, thus confirming the hypothesis of genetic homogeneity of CF previously suggested by the analysis of consanguineous marriages among 624 couples of CF parents. Chi-square test of association indicates a possible linkage disequilibrium between the CF gene and the DNA polymorphism that is most informative in our sample (p*met*H *Taq*I).

INTRODUCTION

The homogeneity of cystic fibrosis (CF) in Italy was indicated by the frequency of consanguineous marriages among 624 couples of CF parents, a result that was more consistent with a single locus hypothesis rather than with a two-loci model [1]. The availability of DNA polymorphisms closely linked to the CF gene on chromosome 7 [2–4] has prompted us to analyze with these markers a sample of Italian CF families previously investigated for consanguinity. With respect to the analysis based on consanguineous marriages [1], linkage analysis represents a more powerful method for testing genetic heterogeneity. The latter method [5] can reveal heterogeneity even when the pattern of segregation of

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[1] Laboratorio di Genetica Molecolare, Istituto Giannina Gaslini, 16148 Genova, Italy.
[2] Centro Regionale Fibrosi Cistica, Verona, Italy.

only a single family is highly discordant from that of the remaining families in the sample considered.

The data collected in the present study on 12 Italian families indicate that they are highly homogeneous with respect to the segregation of the CF gene and of two DNA polymorphic loci located on chromosome 7, thus confirming the anticipation based on consanguinity data [1].

MATERIALS AND METHODS

The diagnosis of CF was documented by a positive sweat test, repeated at least twice, and performed according to the method of Gibson and Cooke [6]. Genomic DNA was extracted from lymphoblastoid lines established for each individual analyzed in this study, according to standard methods [7]. Samples of 5–10 μg of genomic DNA were digested with restriction enzymes, electrophoresed on 0.8% agarose, and blotted on Hybond N filters (Amersham) as described [8]. ^{32}P nick-translated DNA (S.A. 10^8 cpm/μg) from plasmid p*met*D, p*met*H [9], or pJ3.11 [10] was used for hybridization according to Church and Gilbert [11]. Results were usually obtained after 3 days exposure at −80°C on X-Omat filters with double intensification screens. Standard linkage analysis and homogeneity tests were performed using the computer programs LINKAGE [12] and HOMOG [13], respectively.

RESULTS AND DISCUSSION

A sample of 12 two-generation Italian CF families, whose structures are summarized in table 1, were analyzed with the four DNA polymorphisms corresponding to two different loci located on chromosome 7 (table 2). The lengths of the polymorphic fragments revealed by *met* probe and indicated in this table correspond to those recently published [14] rather than to those originally reported [3]. Out of the 12 families examined, eight were informative for three polymorphisms, three for two polymorphisms, and one for only one polymorphism.

Among the 23 affected children of the 12 CF families (see table 1), only one recombinant was found occurring between the CF gene and the *met* locus in the family represented in figure 1. This indicates a close linkage between CF and

TABLE 1

SEGREGATION OF CF IN THE 12 ITALIAN FAMILIES ANALYZED IN THE PRESENT STUDY

s \ r	1	2	3
2	2	—	—
3	—	7	—
4	—	2	1

NOTE: Columns indicate the no. affected children (r) and rows the sibship size (s).

TABLE 2

FREQUENCIES OF FOUR DNA POLYMORPHISMS CALCULATED FROM PARENTAL CHROMOSOMES

LOCUS	PROBE	RESTRICTION ENZYME	NO. CHROMOSOMES	ALLELES (IN kb)	FREQUENCIES	
					This sample	Other studies [14]
met	p*met*H	*Taq*I	44	7.0	.36 (0.072)*	.53
				4.2	.64	.47
met	p*met*H	*Msp*I	28	4.8	.00	.07
				2.3	.43 (0.094)*	.56
				1.7	.57	.37
met	p*met*D	*Taq*I	40	5.0	.88 (0.051)*	.80
				4.0	.12	.20
D7S8	pJ3.11	*Msp*I	38	4.2	.42 (0.080)*	.32
				1.8	.58	.68

* Standard error.

the two polymorphic loci considered in this study. Standard linkage analysis [12] indicates a maximum lod score of 2.52 at $\theta = .042$ (90% confidence interval: .0016, .2067) for *met* locus and a maximum lod score of 3.75 at $\theta = .00$ (90% confidence interval: .00, .089) for locus D7S8. The hypothesis of linkage between CF and either *met* or D7S8 locus as well as that of homogeneity of CF mutation(s) with regard to their location on 7q were further confirmed by the HOMOG test [13], whose results are summarized in table 3.

We also tested the significance of linkage disequilibrium by chi-square test of association in 2 × 2 contingency tables (see table 4). The same test was performed using D7S8 against *met* for the probe-enzyme combination with the most information, that is, p*met*H *Taq*I. The latter polymorphism, which has revealed the highest proportion of informative families in our sample, is the only one that gives an indication of a possible linkage disequilibrium with CF ($P = .088$). Further analysis of the DNA polymorphisms closely linked to CF will allow us to collect a sample of haplotypes representative of the Italian CF population which, by comparison with the "normal" haplotypes, might

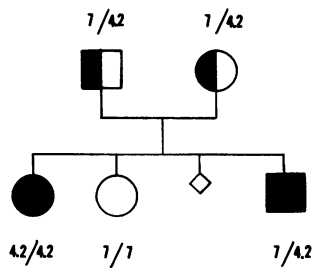

FIG. 1.—Segregation of CF and of polymorphism p*met*H *Taq*I in the only family that shows recombination between the CF gene and one of the two polymorphic loci analyzed. The recombination might have occurred either in a paternal or a maternal gamete and the recombinant might be either one of the affected sibs.

TABLE 3

HOMOGENEITY TEST PERFORMED ON THE 12 CF FAMILIES OF TABLE 1

COMPONENTS OF CHI-SQUARE		D7S8		*met*	
Source	d.f.	Chi-square	*P*	Chi-square	*P*
Heterogeneity	1	0.000	.500	0.000	.500
Linkage	1	51.293	.000	42.087	.000
Total	2	51.293	.000	42.087	.000

yield more definitive evidence in favor or against linkage disequilibrium around the CF locus.

The homogeneity of CF in the Italian population sample analyzed in the present study is clearly indicated by our results, which support a previous suggestion resulting from the analysis of consanguineous marriages [1]. The data obtained so far in different populations based on the analysis of the first DNA polymorphism found to be linked to CF [15] and of other more closely linked polymorphisms [2–4] concordantly support the hypothesis of genetic homogeneity of CF. This conclusion implies homogeneity of mutations with respect to their location on 7q in a limited region of the genome [which might encompass a chromosome length of approximately 10^6 base pairs (bp)] and does not exclude the possibility that other rare genes causing CF might be located on other chromosomes.

Finally, the homogeneity of CF in our population sample has relevant practical implications for the genetic counseling of the Italian families at risk that can now take advantage of the approach based on polymorphic DNA markers for heterozygote detection and prenatal diagnosis.

TABLE 4

ANALYSIS OF LINKAGE DISEQUILIBRIUM BETWEEN THE CF GENE AND FOUR DNA POLYMORPHISMS IN THE 12 FAMILIES OF TABLE 1

	Disequilibrium with CF			
			FREQUENCIES OF RARER ALLELE AT MARKER LOCUS	
POLYMORPHISMS	CHI-SQUARE	*P*	CF chromosome	Normal chromosome
p*met*H *Taq*I	2.92	.088	.47	.21
p*met*H *Msp*I	0.00	...	.34	.34
p*met*D *Taq*I	1.12	.290	.95	.84
pJ3.11 *Taq*I	0.49	.484	.47	.35
	Disequilibrium with D7S8			
p*met*H *Taq*I	0.60	.439	...	...

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