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Genetic Analysis of Hispanic Individuals with Cystic Fibrosis

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Summary

We have performed molecular genetic analyses of Hispanic individuals with cystic fibrosis (CF) in the southwestern United States. Of 129 CF chromosomes analyzed, only 46% (59/129) carry ΔF508. The G542X mutation was found on 5% (7/129) of CF chromosomes. The 3849+10kbC→T mutation, detected primarily in Ashkenazi Jews, was present on 2% (3/129). R1162X and R334W, mutations identified in Spain and Italy, each occurred on 1.6% (2/129) of CF chromosomes. W1282X and R553X were each detected once. G551D and N1303K were not found. Overall, screening for 22 or more mutations resulted in detection of only 58% of CF transmembrane conductance regulator gene mutations among Hispanic individuals. Analysis of KM19/XV2c haplotypes revealed an unusual distribution. Although the majority of ΔF508 mutations are on chromosomes of B haplotypes, the other CF mutations are on A and C haplotypes at higher-than-expected frequencies. These genetic analyses demonstrate significant differences between Hispanic individuals with CF and those of the general North American population. Assessment of carrier/affected risk in Hispanic CF individuals cannot, therefore, be based on the mutation frequencies found through studies of the general population but must be adjusted to better reflect the genetic makeup of this ethnic group. Further studies are necessary to identify the causative mutation(s) in this population and to better delineate genotype/phenotype correlations. These will enable counselors to provide more accurate genetic counseling.

Introduction

Cystic fibrosis (CF) is being recognized with increasing frequency among Hispanic populations. While no Hispanic individuals were identified in a 1989 survey of CF patients, 2.5% of patients were classified as Hispanic in 1990 (Cystic Fibrosis Foundation 1992). A recent estimate of the incidence of CF in Mexico, made by population survey, is 1 in 8,000–9,000 (J. L. Lezana, personal communication). In states of the Southwest, Hispanic individuals account for between 20% and 40% of the population (U.S. Census 1990). For CF clinics and diagnostic laboratories serving Hispanic patients, their genetic characterization is essential for accurate risk assessments and for counseling of families.

We have performed genetic analyses of Hispanic CF patients attending clinics in Albuquerque, Phoenix, Denver, San Diego, and Los Angeles. The geographic origins of this group are Mexico and Central and South America. These individuals were tested for 23 of the more common CF transmembrane conductance regulator (CFTR) gene mutations, on the basis of worldwide frequencies (Tsui 1992). CF chromosomal haplotypes were also determined. The genetic data were correlated with analyses of disease severity.

Patients and Methods

Individuals affected with CF were identified by contacting the CF centers serving southern California and

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the southwestern United States. Hispanic ethnicity was determined by analysis of pedigree data. Those included were, by self-report, of Hispanic heritage. Patients of mixed ethnicity were included in this study if the CF chromosome inherited from the Hispanic parent could be determined. The diagnosis of CF was made by a pediatric pulmonologist. An elevated sweat chloride concentration of >70 mmol/liter was observed in all patients except two who had borderline values. The pediatric pulmonologist believed that these patients fit the typical clinical picture of CF. The clinical data collected included age, height, and weight expressed as percentiles (Health Resources Administration 1976). Pancreatic insufficiency was determined either by clinical history (failure to thrive, chronic diarrhea, greasy floating stools) or by 3-d collection tests of fecal fat (abnormal = fecal fat >7 g/d or plasma immunoreactive trypsin level <10 μg/liter). Pulmonary function tests, including forced vital capacity, forced expiratory volume, and forced expiratory flow, were measured in patients ≥5 years of age.

Venous blood specimens were drawn, and DNA was extracted from white cells by using methods described elsewhere (Kan et al. 1977; Poncz et al. 1982) or by using an ABI automated DNA extractor. The following CFTR gene mutations were identified by published methods: ΔF508 (Rommens et al. 1990); G542X (Kerem et al. 1990); G551D and R553X (Cutting et al. 1990); R1162X (Gasparini et al. 1991); W1282X (Vidaud et al. 1990); N1303K (Osborne et al. 1991); 3849 +10kbC→T (Highsmith et al., submitted); and R117H, Y122X, I148T, 621+1G→T, 711+1G→T, G314E, 1078ΔT, R334W, R347P, Q493X, ΔI507, V520F, 1717 −1G→A, R560T, and 3569ΔC (J. DeMarchi et al., submitted). Alternatively, the G542X, G551D, R553X, and N1303K mutations were assayed by the method of Ng et al. (1991). Haplotypes were determined as described elsewhere (Feldman et al. 1988; Rosenbloom et al. 1989), and the standard letter codes (A, B, C, or D) were assigned (Beaudet et al. 1989).

Results

One hundred twenty-nine chromosomes from Hispanic individuals with CF were screened for 23 of the more common CFTR gene mutations worldwide, including one additional mutation (R1162X) common to Spanish individuals with CF (table 1). The most common CF mutation in Caucasians, ΔF508, was found in 59 (46%) chromosomes. The G551D mutation, present in 3%–4% of CF chromosomes in the general population (Tsui 1992), was not detected in any of the chromosomes in Hispanic individuals. The G542X mutation was found in 5.4% of Hispanic CF chromosomes, similar to the 3% frequency in the general population. This mutation is also common among southern European populations, with a frequency of 12% (Nunes et al. 1991).

Table 1

Mutation Data

Mutation	Frequency in General Population[a] (%)	Frequency (%) in Hispanic Population
ΔF508	67.1	59/129 (46)
G542X	3.4	7/129 (5.4)
3849+10kbC→T	Unknown	3/129 (2.3)
G551D	2.4	0/129 (0)
R553X	1.3	1/129 (.8)
R1162	.85[b]	2/129 (1.6)
R334W	<1	2/129 (1.6)
W1282X	2.1	1/129 (.8)
Other[c]	≤5	0/129 (0)
Undetected	15	54/129 (42)

[a] CF Consortium 1992, unpublished data.
[b] Frequency is as high as 7% in southern Europeans.
[c] Other = ΔI507, 621+1G→T, R117H, N1303K, 711+1G→T, 1717−1G→A, R560T, Y122X, 1148T, G314E, 1078ΔT, R347P, Q493X, V520F, and 3659ΔC.

The 3849+10kbC→T mutation, which was detected in 3 of 129 chromosomes, has been detected primarily in the Ashkenazi Jewish population. In particular it has been found in 8 of 13 individuals affected with CF but who have normal sweat chloride values (Highsmith et al., submitted). Two of the three patients in our study who carry this mutation also had normal sweat chloride tests.

The R334W mutation, found in 2 (1.6%) of 129 chromosomes in our study, was first identified in southern European populations and makes up ~1% of CF mutations in this group (Gasparini et al. 1991).

The R1162X mutation, which was first identified in Spanish individuals (Gasparini et al. 1991), was also detected in our population in 2 (1.6%) of 129 chromosomes. It has a high frequency (7%) among southern European populations, as well as among certain Native American populations of the Southwest (Mercier et al. 1992).

Three other mutations—R553X, W1282X, and N1303K—are rare in many populations, and any deviation from expected frequencies cannot be determined from our study.

Table 2

Chromosomal Haplotype Summary

Chromosome Type	No. (%) of Haplotypes				Total
	A	B	C	D	
CF:					
ΔF508	2 (4)	36 (72)	5 (10)	7 (14)	50
Non-ΔF508[a]	12 (29)	14 (33)	14 (33)	2 (5)	42
Normal[b]	22 (49)	5 (11)	16 (34)	4 (9)	47

[a] Includes chromosomes carrying either G542X, R1162X, W1282X, R334W, R553X, 3849+10kbC→T, or an unidentified mutation.

[b] Non-CF chromosomes were identified in the parents of affected individuals; both parents were not available in all cases.

Haplotypes of CF chromosomes were determined by analysis of two linked markers, XV2c/*Taq*I and KM19/*Pst*I (Beaudet et al. 1989) (table 2). For some individuals the phase of the markers could not be determined, and the haplotypes could not be assigned. The ΔF508 mutation was found predominantly on chromosomes of B haplotype.

Clinical data, including height and weight percentiles, lung function, and pancreatic insufficiency were analyzed for the Hispanic individuals. Comparison of age at examination, growth parameters, and tests of pancreatic and pulmonary function did not reveal any statistically significant differences from the general CF population.

Discussion

The genetic profile of the Hispanic CF population in the southwestern United States differs from the general U.S. population with CF. The frequency (46%) of the ΔF508 mutation in the Hispanic population is significantly lower ($\chi^2 = 8.2$, $P \leq .005$) than the >70% frequency found among the general CF population in the United States (Cystic Fibrosis Genetic Analysis Consortium 1990), which is largely of northern European origin. The ΔF508 frequency in southwestern Hispanics is equivalent to that of southern European countries. It is also similar to that reported in Mexico, where 30% of chromosomes from 74 living CF patients and 55% of chromosomes from 32 autopsy specimens carried the ΔF508 mutation (Orozco et al. 1993). The absence of the N1303K mutation among Hispanic patients distinguishes this group from those of southern Europe, where it is found on >6% of CF chromosomes (Nunes et al. 1991). The total frequency of detectable mutations among the Hispanic CF patients was 58%. This is significantly lower ($\chi^2 = 8.6$, $P \leq .005$) than that of the general U.S. population, where screening for this same set of mutations allows detection of ~85% of mutations (Cystic Fibrosis Genetic Analysis Consortium, unpublished data). This may indicate that there are mutations of Native American origin in this group.

The haplotype distribution also shows features that differ from those of the overall U.S. population. The percentage of chromosomes with the ΔF508 mutation that are of B haplotype shows an ethnic bias; it accounts for 90% or more of ΔF508 chromosomes in northern European countries but for only 80% or less in many countries of southern and eastern Europe (European Working Group in CF Genetics 1990). This latter figure is similar to the 72% frequency seen in our population. As with the ΔF508 mutation, the frequency of the B haplotype among non-ΔF508 mutations is also higher than the frequency of this haplotype on normal chromosomes. This has been observed elsewhere (European Working Group in CF Genetics 1990), and several mutations, including G542X and G551D, have been identified on chromosomes of this haplotype (Kerem et al. 1990). The A and C haplotypes occur more frequently in the Hispanic group, on both ΔF508 and non-ΔF508 chromosomes (on 62% of Hispanic CF non-ΔF508 chromosomes vs. 41% reported in European populations [European Working Group in CF Genetics 1990]). The appearance of ΔF508 on non-B haplotype chromosomes in this population is likely the result of recombination between the linked markers and the mutation, as happens in a population where the mutation is more ancient. Thus, the Hispanic group resembles the populations found geographically close to where the ΔF508 seems to have originated.

When taken together, the genotype and haplotype data reveal significant differences between individuals with CF of northern European descent and those of Hispanic descent from the southwestern United States. Further genetic analysis is necessary to identify the causative mutation(s) in this group, as well as to allow better genotype/phenotype correlations. This information is essential for accurate genetic counseling as well as for prognostication of disease severity for these individuals.

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