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Screening for Five Mutations Detects 97% of Cystic Fibrosis (CF) Chromosomes and Predicts a Carrier Frequency of 1:29 in the Jewish Ashkenazi Population

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Summary

To determine the distribution and frequency of cystic fibrosis (CF) mutations in the Israeli population, we have screened 96 patients for 11 relatively common mutations. Five mutations—ΔF508, G542X, W1282X, N1303K, and 3849 + 10kb C→T—were found to account for 97% of the CF alleles in the Ashkenazi Jews. In contrast, of the 11 mutations tested, only ΔF508 was detected in Jewish patients of Sephardic or Oriental origin, accounting for 43% of the CF alleles. Four mutations—ΔF508, G542X, W1282X, and N1303K—accounted for 55% of the CF alleles in Arab patients. In a pilot screening study, a random sample of 424 Ashkenazi individuals was analyzed for three mutations—ΔF508, W1282X, and G542X. Thirteen individuals were detected as heterozygotes (six for ΔF508 and seven for W1282X), predicting a heterozygote frequency of 1:29. This is similar to the frequency of carriers in the Caucasian population of northern European ancestry. On the basis of these data, the Ashkenazi population is considered to be a candidate for CF heterozygote screening.

Introduction

Cystic fibrosis (CF) is the most common recessive disorder in Caucasian populations of northern European ancestry, affecting 1:1,600–1:4,000 live births (Boat et al. 1989). Heterozygote detection based on the protein product of the CF gene is not yet available. Cloning of the CFTR gene (Riordan et al. 1989; Rommens et al. 1989) and identification of mutations in CF patients (Kerem et al. 1989, 1990; Cutting et al. 1990; Davies 1990) have made it possible to directly diagnose heterozygotes by mutation analysis. This approach allows the identification of couples of a 1-in-4 risk, before the first affected child is born and regardless of their family history. However, the yield of carrier detection by mutation analysis depends on the fraction of known mutations in a given population (Beaudet 1990).

In North America and northern Europe the ΔF508 mutation accounts for about 70% of the CF mutations (Kerem et al. 1989; Lemna et al. 1990; Romeo and Devoto 1990). Many mutations have been identified on the remaining CF chromosomes, each of which accounts for only a minor fraction. The total fraction of already identified mutations varies from 85%–87% in North America and Europe (Ng et al. 1991; Simon-Bouy et al. 1991; Cutting et al. 1992) to 60%–70% in southern Europe (Devoto et al. 1991; Nunes et al. 1991). Only in discrete populations such as the Ashkenazi Jews (Cutting et al. 1992; Lerer et al. 1992; Shoshani et al. 1992) and the French-Canadians (Rozen et al. 1992) have predominant mutations, other than the ΔF508, been identified.

In order to design a heterozygote screening program in a given population, two sets of data are of crucial importance: (1) the type of mutations and their proportion in the CF patients and (2) the heterozygote

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frequency in the general population. The aim of the present study was to approach these two prerequisites, with a special emphasis on the Ashkenazi community.

Patients and Methods

Patients

Ninety-six CF patients of unrelated families referred to us, during the last 6 years, from various medical centers in Israel have been partially described elsewhere (Lerer et al. 1992). This group includes 73 Jewish patients: 45 of Ashkenazi origin, 15 of non-Ashkenazi origin (from countries around the Mediterranean and from Iran and Iraq), and 13 of mixed origin (table 1). The group of Arab patients is heterogeneous and includes Moslem, Christian, Druze, and Bedouin families. In case of consanguinity, only one CF chromosome per family was counted, although we are aware of the fact that in a common disease the affected offspring is not always homozygous by descent. A total of 94 CF chromosomes were of Ashkenazi origin, 37 were of non-Ashkenazi origin, 7 were from Jews from Turkey, and 31 were of Arab origin (table 1).

Blood samples of 424 anonymous healthy Ashkenazi individuals were tested for CF mutations. These individuals were healthy and represent a random sample of the Ashkenazi individuals ascertained through a screening program for other genetic disorders (e.g., Tay-Sachs disease).

DNA Preparation and PCR Amplification

Genomic DNA was extracted from blood by a modification of the procedure described by Miller and Polesky (1988). Alternatively, DNA was extracted by the short procedure described by Kawasaki (1990). The regions encompassing the mutations of interest were simultaneously amplified by PCR using DNA primers: C16B and C16D for ΔF508 (Kerem et al. 1989), 11i5 and 11i3 for G542X, 20i5 and 20i3 for W1282X, and 21i5 and 21i3 for N1303K (Kerem et al. 1990; Vidaud et al. 1990; Osborne et al. 1991; Zielenski et al. 1991*b*). The DNA primers for the mutation 3849 + 10kb C→T were forward (5′-AGGCTTCTCAGTGATCTGTTG-3′) and reverse (5′-GAATCATTCAGTGGGTATAAGCAG-3′) (E. W. Highsmith, personal communication). Approximately 100 ng of genomic DNA, 0.2 mM of each dNTP, 0.001 mM of the primers, and 2 units of *Taq* polymerase (Cetus) were amplified in standard PCR buffer (Cetus) for 30 cycles—each at 58°C for 30 s, 72°C for 90 s, and 92°C for 30 s—followed by a final extension at 72°C for 10 min. Ten microliters of the PCR product was electrophoresed on 3% Nusieve (FMC Bioproducts)–1% agarose gel to confirm the amplification of the three fragments.

Mutation Detection

Mutations ΔF508 and G542X were detected by using oligonucleotides oligo-N, oligo ΔF, 542NL, and 542X according to a method described elsewhere (Kerem et al. 1989 and 1990). Mutation W1282X was detected by oligonucleotide 1282X (5′-CAACAGTGAAGGAAAGCCTT-3′), while the oligonucleotide 1282NL (5′-CAACAGTGGAGGAAAGCCTT-3′), was used to detect the normal allele. Allele-specific oligonucleotide hybridizations and autoradiography

Table 1

Distribution of CF Chromosomes, by Ethnic Group

Origin	No. of Patients	No. of CF Chromosomes in			
		Jews			
		Ashkenazi	Non-Ashkenazi	Turkish	Arabs
Jewish:					
Ashkenazi	45[a]	86			
Non-Ashkenazi	15[b]		26		
Ashkenazi × non-Ashkenazi	6	6	6		
Ashkenazi × Turkish	2	2		2	
Non-Ashkenazi × Turkish	5		5	5	
Arab	23[b]				31
Total	96	94	37	7	31

[a] In four Ashkenazi patients, only one CF chromosome was counted, because of consanguinity in one case, uniparental disomy in one case (Voss et al. 1989), and parental marriage with a non-Jewish partner in two cases.

[b] In 4 non-Ashkenazi and 15 Arab patients, only one CF chromosome was counted, because of consanguinity.

were performed according to methods described elsewhere (Lerer et al. 1992). The hybridization and wash conditions were as follows: W1282X—hybridization at 37°C for 60 min and three washes in 2 × SSC, 0.1% SDS (twice at room temperature for 10 min and then at 59°C for 10 min); ΔF508—hybridization 37°C for 60 min and then three washes in 2 × SSC, 0.1% SDS (twice at 37°C for 10 min and then at 42°C for 10 min); G542X—hybridization at 42°C for 2 h and then two washes in 2 × SSC, 0.1% SDS (once at 42°C for 10 min and once at 45°C for 10 min). The W1282X mutation was also identified by cleavage of the PCR product with *MnlI* (Vidaud et al. 1990). The mutation N1303K was detected by PCR-mediated site-directed mutagenesis as described by Friedman et al. (1991). The same approach was used as an alternative method to detect ΔF508 and G542X. For ΔF508 analysis, *Dpn*II digestion was performed instead of *Mbo*I, as suggested by Friedman et al. (1990). The mutation 3849 + 10kb C→T was detected by cleavage of the PCR product by *Hph*I (E. W. Highsmith, personal communication). The mutations G551D, S549N, S549I, S549R, and 1717-1 G→A were analyzed according to the method of Kerem et al. (1990), R553X according to the method of Cutting et al. (1990), and 621 + 1 G→A according to the method of Zielenski et al. (1991*a*).

Results

Five mutations—W1282X, ΔF508, G542X, N1303K, and 3849 + 10kb C→T—were found in our patients. The distribution of these mutations differed by ethnic group (table 2). The mutations 621 + 1 G→T, 1717-1 G→A, S549N, S549I, S549R, G551D, and R553X were not found in our patients. Among the Ashkenazi, three mutations—W1282X (48%), ΔF508 (30%), and G542X (12%)—accounted for 90% of the CF mutations, while mutations N1303K and 3849 + 10kb C→T were found on an additional 7% of CF chromosomes. In the non-Ashkenazi Jews, the ΔF508 mutation accounted for 43% of the CF mutations; however, the W1282X (3%) mutation was identified on only one CF chromosome, leaving 54% of the CF mutations unidentified. The CF chromosomes of Turkish origin carried the same mutations as did the Ashkenazi group and were therefore classified separately from the non-Ashkenazi group. In the Arab patients, four mutations—ΔF508, N1303K, W1282X, and G542X—were found, accounting for 55% of the CF mutations. The N1303K mutation was more frequent in the Arabs than in the Jewish patients.

To evaluate the gene frequency in the Ashkenazi population, a random sample of 424 Ashkenazi individuals (848 chromosomes) was screened for the presence of the three mutations W1282X, ΔF508, and G542X. Thirteen individuals were identified as heterozygotes—six with the ΔF508 mutation and seven with the W1282X mutation. In this sample we did not identify any carrier having the G542X mutation.

Discussion

The predominant mutation in Caucasians, ΔF508, was found in all ethnic communities in Israel but at a significantly lower frequency (31%) than in northern

Table 2

Distribution of CF Mutations, by Ethnic Group

	Frequency (no.) in			
	Jews			
Mutation	Ashkenazi (n = 94)	Non-Ashkenazi (n = 37)	Turkish (n = 7)	Arabs (n = 31)
F508	.30 (28)	.43 (16)	.29 (2)	.26 (8)
G542X	.12 (11)		.43 (3)	.03 (1)
W1282X	.48 (45)	.03 (1)	.14 (1)	.10 (3)
N1303K	.03 (3)			.16 (5)
3849 + 10kb C→T	.04 (4)		.14 (1)	
Subtotal	.97	.46	1.00	.55
Unidentified mutations	.03 (3)	.54 (20)	0	.45 (14)

Note.—n = number of CF chromosomes.

European and North American populations. The low frequency of ΔF508 in Jewish patients as well as in southern Europe is well documented (European Working Group on CF Genetics 1990; Lemna et al. 1990; Lerer et al. 1990). This indicates that the populations in Israel are genetically distinct even though the ΔF508 mutation may have a common ancestral origin in all populations.

On the basis of the distribution of five mutations, it is clear that the Ashkenazi are distinct within the Jewish population. Ninety-seven percent of the Ashkenazi CF mutations are accounted for by the five mutations, the highest proportion in any population. The profile of the CF mutations in Turkish Jews is similar to that of the Ashkenazi CF mutations, consistent with our previous hypothesis that CF in Turkish Jews is the result of a founder effect from Ashkenazi immigrants (Lerer et al. 1992). In non-Ashkenazi and Arab patients, most of the CF mutations remain unknown. Because of haplotype heterogeneity, we expect to find many mutations, each of which will account for a small fraction of CF mutations.

Our data indicate that the frequency of CF in the Ashkenazim is higher than that in the non-Ashkenazim. This impression is based on a twofold excess of Ashkenazi CF chromosomes compared with CF chromosomes from non-Ashkenazi groups, while the proportion of each group is almost equal in the general population. An ascertainment bias due to socioeconomic and geographical background is unlikely, since our patients were referred from all the medical centers in Israel and since the compliance of the families was almost complete.

The CF heterozygote frequency in the Ashkenazi population in Israel has been estimated from two epidemiological surveys. In 1963, Levin (1963) concluded, on the basis of 14 cases born to Ashkenazi parents, that the CF gene frequency in the Ashkenazim is 1:70. This frequency is lower than the 1:50 estimated for Caucasians living in Europe and North America (Tsui and Buchwald 1991). Katzenelson and Ben-Yishay (1979) claimed that the incidence of CF in the Ashkenazim is the same as in European populations, but, because of underdiagnosis, they could not estimate the gene frequency.

To estimate the gene frequency in the Ashkenazim, we chose the approach of mutation analysis, to avoid problems due to misdiagnosis, late diagnosis, and incomplete ascertainment. We screened for three mutations—ΔF508, W1282X, and G542X—which are expected to detect 90% of the carriers. Our sample included 848 CF transmembrane-conductance-regulator alleles of Ashkenazi origin. In this sample, 13 individuals were detected as heterozygotes for either the W1282X mutation or the ΔF508 mutation. If these mutations represent 90% of the CF mutations in the Ashkenazi community, then the actual frequency of CF mutations is 1:58 chromosomes, predicting a heterozygote frequency of 1:29 (95% confidence internal for point estimation of the rate of CF carriers in the Ashkenazi population is .0168–.0512). Since this is a small sample of the Ashkenazi population, the value of 1:29 can serve as an estimate for risk calculation until data based on a larger populations sample are available. These data indicate that CF is one of the most frequent lethal inherited disorders in the Ashkenazi community (as is Tay-Sachs disease) and that the CF carrier frequency in the Jewish Ashkenazi community is close to that in Caucasians living in northern European and North American countries.

With the mutation distribution and approximate mutant-gene frequency established for the Ashkenazi population, the efficacy of a heterozygote-screening program can be evaluated. Screening for the five mutations will detect 94% of Ashkenazi couples at 1-in-4 risk for an affected child. An individual who is not a carrier of any of the five mutations has a risk of 1:933 of being a carrier. If both partners do not carry one of the five mutations, their risk for an affected child is 3.4×10^{-6}. If only one partner is tested and is not a carrier of any of the five mutations, then the risk for an affected child is 1×10^{-5}. In the 6%–7% of couples where one of the partners is a carrier and the other partner is not a carrier of any of the five mutations, the risk for an affected child is 1:3,732. This risk is very similar to the 1:3,364 prior risk. Therefore, screening of the Ashkenazi population for five CF mutations will identify the vast majority of couples who are at 1:4 risk, significantly increasing their risk, from 1:3,364 to 1:4. For couples where neither is a carrier, the risk will be significantly lowered, to approximately 1:3,000,000. Couples where only one member is identified as a carrier will have no change in risk status.

An NIH Advisory Panel (Workshop on Population Screening for the Cystic Fibrosis Gene 1990) advised waiting until the mutation detection rate was 95% or greater before any general screening of the U.S. Caucasian population take place. The large number of mutations and lower detection rates in the U.S. population indicate that 95% will not be easily achieved. However, our data suggest that carrier screening in the Ashkenazi population may be feasible

now, since (1) only five mutations need to be screened to detect the vast majority of CF chromosomes; (2) the carrier frequency in this population is high enough to warrant such a screening program; (3) the detection rate is high enough to provide a high benefit-to-risk ratio; and (4) the Ashkenazi population is a sufficiently discrete population in which it should be possible to correctly identify the ethnicity of the majority of participants.

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