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Cystic Fibrosis Heterozygote Screening in the Orthodox Community of Ashkenazi Jews: The Dor Yesharim Approach and Heterozygote Frequency

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Key Words

Cystic fibrosis
Heterozygote screening
Heterozygote frequency

Abstract

In the community of the Orthodox Jews most of the marriages are arranged; a screening program that is aimed at preventing the marriage of two carriers of autosomal recessive disorders is conducted by the Dor Yesharim organization. A random sample of 6,076 individuals of the Orthodox Jewish Ashkenazi community, were screened for the five mutations common in Ashkenazi patients ($\Delta F508$, W1282X, G542X, N1303K, 3849+10Kb C→T). Two hundred thirty-two carriers were identified, giving a heterozygote frequency of 1:26. The relative frequencies of the individual mutations in the general population were comparable to those in the patients.

Introduction

Genetic screening programs of the general population are aimed at preventing common genetic diseases. There are several approaches for conducting genetic screening with depend on the target population and the type of the disease [1]. Genetic screening is offered to populations at high risk for autosomal recessive disorders such as Tay-Sachs disease [2], hemoglobinopathies (sickle cell anemia or thalassemia) and lately for cystic fibrosis (CF) [3]. The strategy of heterozygote screening varies; in most centers one partner is tested and if a carrier is found, the spouse is tested after proper counseling. In couples at a 1:4 risk for an affected child the option of prenatal diagnosis is offered.

In autosomal recessive disorders in which the screening test is based on mutation analysis, the carrier rate detection depends on the pool size of the mutations and the fraction of known defective alleles in the population. Therefore, in couples who undergo mutation analysis in a screening test with one of the partners identified as a heterozygote, the residual risk cannot be reduced and the anxiety of the couple cannot be alleviated [4]. In order to avoid the anxiety, Wald [5] and Livingstone et al. [6] suggested a new approach of screening, in which both partners are the unit of the screening and only in cases where both of them are identified as carriers are they informed. Couples with one heterozygote partner are not informed.

In the Ashkenazi Jews, 97% of the CF mutations are accounted for by five mutations [7], allowing a detection

of 94% of the couples at a 1:4 risk for an affected child; therefore in this subpopulation of the Jewish people a screening test is offered.

In the orthodox community of the Ashkenazi Jews, a unique screening approach is being implemented which meets the religious restrictions relating to family planning, and the prohibition on pregnancy termination. In this community most of the marriages are arranged, and therefore the screening program is aimed at preventing the marriage of two carriers. Dor Yesharim (meaning righteous generation) is a non-profit organization established in 1985 in order to screen the religious youth before engagement. The organization started with Tay-Sachs screening [8], and since then, CF and Canavan's disease have been introduced into the screening program. There is a similarity in this strategy of screening to that of couples screening [6], although the reasoning is different; in both approaches couples with one heterozygote partner are not informed. The experience gained by Dor Yesharim is impressive; the compliance rate is over 90% and hardly any affected children with Tay-Sachs disease were born in this community in the last 5 years.

We wish to present our results of the CF heterozygote screening conducted by the Dor Yesharim organization in Israel, and, based on this data, the CF heterozygote frequency and the frequency of each of the individual mutations in the Jewish Ashkenazi population that belong to the Dor Yesharim community are also presented.

Subjects and Methods

The Population

A total of 6,076 Ashkenazi Jews aged <18 years, males and females in approximately equal numbers, were ascertained without any preselection as to their family history or any other criteria. Each examinee was informed about the aim of the test, and for confidentiality each person was assigned a number that was kept by Dor Yesharim as the only means of identification. The personal number is used in due time, before the marriage of a couple is arranged, to identify the screening results.

Mutation Analysis

DNA was extracted from 200 μ l of thawed blood in a short protocol [9]. The five mutations common in Ashkenazi Jews [7] (Δ F508, W1282X, G542X, N1303K and 3849+10Kb C \rightarrow T) were analyzed by one of the following methods: restriction enzyme analysis using site-directed mutagenesis [10], and the ARMS method [9] for the individual mutations, and in the last 500 samples a clinical trial of the CF 12-A kit tests (Zeneca, Cellmark Diagnostics) was performed in parallel with restriction analysis (fig. 1). Quality control was performed by Dor Yesharim coordinators by introducing known heterozygotes to their samples.

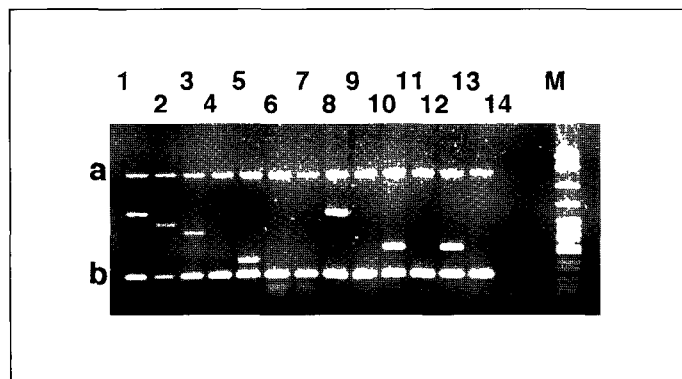


Fig. 1. The mutation analysis using the CF12-A kit tests. The upper (a) and lower (b) fragments are PCR products of genomic regions not related to the CFTR gene and are used as a control for the PCR efficiency in the test. If the large PCR product is not seen, the test cannot be read. Each lane represents a test of one individual with the following genotypes: lanes 1 and 8, G542X/+; lane 2, W1282X/+; lane 3, N1303K/+; lanes 4, 6, 7, 9, 11 and 13, +/+; lane 5, 3849+10Kb C \rightarrow T; lanes 10 and 12, Δ F508; lane 14, no DNA, control.

Results

In the random sample of 6,076 individuals, 232 individuals were found to be carriers (table 1), giving a heterozygote frequency of 1:26 (1:23–1:30, 95% confidence interval for the rate of heterozygotes 0.0382 is 0.0048). The 95% confidence interval for the rate 1:48 (0.02) of W1282X was 1:43–1:61 (0.0235–0.0165), and for the rate 1:78 of Δ F508 it was 1:48–1:200 (0.021–0.005). The relative proportion of each of the five mutations was compared to their relative proportion in the Israeli patients [7, 11]; the mutations Δ F508, W1282X, N1303K and 3849+Kb C \rightarrow T were found in similar relative frequencies in the patient group. A deviation was noted in the frequency of the mutation G542X, being 5.17% in the general population of Dor Yesharim and 12.1% [7] or 9.6% [11] in the patient group.

The CF12-A kit test includes the mutation 1717-1 G \rightarrow A; none of the 500 tests gave a positive result for this mutation.

To date only a part of the individuals screened in this study were engaged. Therefore, the number of couples whose engagements were annulled on the ground that both of them were heterozygotes cannot be assessed at present.

Table 1. Heterozygote frequency of the five common CF mutations in 6,076 Ashkenazi individuals

	Δ F508	W1282X	G542X	N1303K	3849+ 10Kb C→T	Total
Heterozygotes, n	78	126	12	11	10	232
Relative % in population	31.46	54.31	5.17	4.74	4.31	100
Relative % in patients	30.7 ¹	49.4 ¹	12.1 ¹	3.3 ¹	4.4 ¹	100
	29.8 ²	51.1 ²	9.6 ²	3.3 ²	6.4 ²	
Frequency in population	1:78 ³	1:48 ⁴	1:506	1:552	1:607	1:26 ⁵

¹ According to Abeliovich et al. [7]

² According to Kerem et al. [11].

³ 95% confidence interval 1:48–200.

⁴ 95% confidence interval 1:43–61.

⁵ 95% confidence interval 1:23–30.

Discussion

In our previous study [7], based on 424 individuals, the frequency of CF heterozygotes was 1:29; however, in order to obtain an accurate figure a greater sample should be analyzed. The present study includes a sample of 6,076 individuals and 1:26 were CF heterozygotes for any of the five mutations. Since the five mutations comprise 97% of CF mutations in Ashkenazi patients, the corrected rate for CF heterozygote frequency is 1:25.

The relative frequencies of the mutations W1282X, Δ F508, N1303K and 3849+10Kb C→T in the patient group [7, 11] and in the general population are comparable; the mutation G542X was more frequent in the patient group (12.1 or 9.6%) than in the screened group (5.17%). The similarity between the mutation distribution in the general population and that in the patient group indicates the following: (1) Gametes carrying each of the mutations have equal probability to participate in fertilization and to give rise to a viable fetus. This conclusion is in contrast to what was suggested by Kalman et al. [12], who analyzed the screening results of 1,946 individuals and concluded that the discrepancy observed between the relative frequencies of the mutations W1282X and Δ F508 in the patient group and in the general population were of biological significance. In the study of Kalman et al. [12] the frequency of Δ F508 was 1:55, lower than the 1:31 resulting in the present work for Δ F508. This discrepancy could be due to non-randomness of the sample and/or deviation due to sample size that could mislead the conclusion (the 95% confidence interval for the rate 0.018, the frequency of W1282X and of Δ F508 in the study of Kalman et al. [12], was 0.024–0.012, namely,

1:41–1:83). (2) The four mutations W1282X, Δ F508, N1303K and 3849+10Kb C→T are distributed equally among the subgroups of the Ashkenazi populations. The mutation G542X may be more common in some groups of Ashkenazi Jews that are underrepresented in the population of the Orthodox community. The communities that Dor Yesharim screens are mainly Ashkenazi Jews of Central European origin (Hungary, Czechoslovakia, Latvia and Poland), and the patient group was of the general Ashkenazi population (Eastern, Central and Western European origin). However, it should be emphasized that in order to evaluate accurately the frequency of a mutation with an expected frequency of 1:506 (95% confidence interval for the rate of G542X carrier 0.002 is 0.013) in the general population, the size of the sample should be increased.

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