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Screening for Carriers of Cystic Fibrosis among Pregnant Women: A Pilot Study

Key Words
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Prenatal diagnosis

Abstract
Cystic fibrosis (CF) is one of the most common hereditary disorders among Caucasians. In such populations a highly prevalent mutation causing CF, ΔF508, has been identified. It comprises 88% of Danish CF mutations. This mutation and five others account for 90% of all CF mutations, making carrier screening on a population basis worthy of consideration. We have therefore performed a pilot screening programme for CF carriers among pregnant women. From June 1, 1990, for the following 2 years, 6,599 women were tested: 172 were heterozygous for ΔF508. Three of 162 partners tested were also heterozygous for ΔF508. After genetic counselling all three couples at risk for having a child with CF requested prenatal diagnosis. One fetus was homozygous for ΔF508, and the pregnancy was terminated. With currently available techniques, the screening programme presented here causes no practical problems, and screening for CF carriers can easily be run on a larger scale.

Introduction

Cloning of the cystic fibrosis (CF) gene and the subsequent identification of a highly prevalent mutation, ΔF508, have made carrier screening worthy of consideration in several countries [1–3]. Such screening could give couples with a high risk of having a child with CF a reproductive choice which otherwise would be restricted to families with at least one member with CF. However, the frequency of ΔF508 varies greatly among different populations [4], influencing the percentage of carriers detected when screening for this mutation alone. It has been argued that a carrier detection rate $\geqslant 90\%$ must be obtained before a carrier screening programme can be recommended [5].

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Table 1. Mutations detected in 285 unrelated CF patients of Danish origin [7]

Mutation	n	%
ΔF508	500	87.7
G542X	4	0.7
G551D	1	0.2
N1303K	5	0.9
621+1G→T	4	0.7
W1282X	1	0.2
Unknown	55	9.6
Total	570	100.0

We have shown previously that approximately 90% of CF carriers in the Danish population can be detected by testing for ΔF508 [6, 7]. Therefore, we have performed a pilot screening programme among pregnant women with the aim of assessing the practical and economic aspects of such a programme. In addition to data relevant to these goals, our results allow a comparison between the expected and the observed frequencies of CF carriers.

Methods and Target Population

Several criteria must be fulfilled before starting a screening programme for CF: (1) the approximate incidence of the disorder must be known in the population studied; (2) accurate estimates of the frequencies of the most common CF mutations should be made; (3) easy and cost-effective methods for collecting samples and for analysis of the mutation(s) must be available; (4) counselling about the risk of CF before and after the test must be available from experienced personnel, and (5) the couples identified as carriers must have access to prenatal diagnosis and termination of affected pregnancies.

These criteria were met as follows:

(1) The incidence of CF in Denmark has recently been estimated to be 1:4,700 [8] giving an overall mutant gene frequency (q) of 1:68 and a carrier frequency (2pq) of 1:34.

(2) Of 570 investigated chromosomes from 285 unrelated Danish CF patients, 500 (88%) were found to carry ΔF508 [6]. Among the 70 chromosomes without ΔF508, five other known CF mutations were identified on 15 chromosomes: G542X, 621+1G→T, G551D, N1303K, and W1282X [7, 9–12] (table 1).

(3) The ΔF508 mutation was detected by the polymerase chain reaction (PCR), using primers flanking the mutation, separation of the PCR product(s) by polyacrylamide gel electrophoresis, and direct visualization by ethidium bromide staining [13]. The analysis was performed on 3 μl whole blood with no purification step prior to amplification of the DNA fragment(s) containing ΔF508. The other five CF mutations were detected by PCR using primers flanking the appropriate exons, followed by restriction enzyme cleavage [11–13] or by using the commercially available assay based on the amplification refractory mutation system (ARMS) for detecting ΔF508, G542X, G551D and 621+1G→T (courtesy of Cellmark [14]). Positive test results were confirmed by repeated analysis.

(4) and (5) Facilities for genetic counselling and prenatal diagnosis are well established in our centre. Records of test results are kept in the womens' hospital records, which are confidential.

Target Population

The following two groups were chosen as the target population for the pilot study. Group 1: Pregnant women referred to our hospital for chorionic villus sampling (CVS) and prenatal chromosome analysis, most often because of advanced maternal age.

Group 2: Women attending the obstetric outpatient clinic for routine prenatal care.

None of the participating women had a family history of CF.

Risk Calculations

The carrier detection rate of the method (sensitivity) was 88% for ΔF508 alone and 90% when five other mutations were also considered [7]. With a CF carrier frequency of 1:34, the rate of false-negative results of carrier testing is $2pq(1 - 0.88)/(p^2 + 2pq(1 - 0.88)) = 1:284$, and for a detection rate of 90%, 1:341 [15]. The various risks for a couple having a child with CF after carrier testing, depending upon the test result, are given in table 2.

Table 2. Risk of having a child with CF

	Partner not tested	Partner negative[1]
Woman, ΔF508 negative	1 in 38,624	1 in 387,376
Woman ΔF508 positive	1 in 136	1 in 1,364[3]
Fetus positive[2]	1 in 68	1 in 682[4]
Fetus negative	0	0

1 Negative for ΔF508, and 5 other mutations.
2 The fetus was only tested in group 1 and only when the woman was positive (carrier) for ΔF508. Provided that she does not have CF, the risk for the fetus, negative for Δ508, will be zero.
3 1 × 1/341 × 1/4.
4 1 × 1/341 × 1/2.

A leaflet explaining the purpose of the pilot programme, including information about CF, was sent to all women in a letter with their first appointment to the clinic. Written informed consent was obtained. All participating women were informed in writing about the result of their analysis. If a woman was ΔF508-positive, she was informed both by mail and by telephone about the result and the offer was made to have her partner tested as soon as possible for ΔF508, G542X, G551D, N1303K, 621+1G→T, and W1282X. If the partner was found to carry one of these mutations, the couple was offered further genetic counselling, including the possibility of prenatal diagnosis. If the partner did not have one of the mutations tested for, the couple was informed by telephone that the risk of having a CF child was very low, albeit not zero.

Results

In the period June 1, 1990 to June 1, 1992, 7,400 women were asked to participate in the programme. More than 98% and 80% of the women in groups 1 and 2, respectively, wished to do so. The average maternal and gestational ages in the two groups are shown in table 3. 6,599 women were tested for ΔF508. The average duration of analysis (the time period from blood sampling to mailing the first answer) was 6 days. The time period which elapsed from testing the partner until delivering the final answer for the couple was 11 days (table 3).

We identified 172 women as heterozygotes for ΔF508 (table 4). All but 10 of the 172 partners were tested, revealing three ΔF508 heterozygous men (table 4). No partner was found to be heterozygous for any of the other five mutations. Thus three couples at risk for having children with CF were identified. The three women came from group 1 (ages 21, 23 and 38 years). After genetic counselling all three couples requested prenatal diagnosis. This was done on chorionic villus cells already obtained for other reasons. The first fetus examined was homozygous for ΔF508, and the couple chose to terminate the pregnancy. The other two fetuses were heterozygous for ΔF508, and the pregnancies continued.

Table 3. Age at referral, gestational age, and test result delay in groups 1 and 2

	Group 1 (n = 3,545)		Group 2 (n = 3,054)	
	median	range	median	range
Age at referral, years	33	19–47	28	17–41
Gestational age, weeks	11	8–15	13	11–20
Time delay for women, days[1]	6	4–11	6	4–11
Time delay for couples, days[2]	11	4–28	11	4–28

[1] Time delay between first blood sampling and result of the woman's CF screening test.
[2] Time delay between first blood sampling of the woman and her partner's result.

Table 4. CF screening results

Persons tested	n	N/ΔF508[1]	ΔF508/ΔF508[2]
Pregnant women	6,599	172	0
Partners	162	3	0
Fetuses (group 1)	96	44	1[3]

[1] Heterozygotes for ΔF508.
[2] Homozygotes for ΔF508.
[3] Pregnancy terminated.

Discussion

Although recent advances in the treatment of CF allow survival for three or even four decades, more than 95% of Danish CF families with a previous child with CF have requested prenatal diagnosis in subsequent pregnancies [Danish CF Centre, pers. commun.].

Many arguments have been presented for and against population-wide carrier screening for CF [16–18]. Only pilot studies such as ours and the studies recently published [17, 19] can reveal if it is possible to prevent the birth of children with CF without insurmountable medical, ethical, legal, and social problems. We decided to offer the screening to pregnant women for two reasons: it would have immediate consequences for the couples involved, and it is the most economic procedure; i.e., screening all fertile women would result in unnecessary examinations as not all women become pregnant. Furthermore, in our experience, most couples are not concerned about their genetic load before pregnancy. Even in well-informed families with X-linked disorders, it is our experience that more than 90% are not referred to our clinic until pregnancy has occurred.

The patients' acceptance rate of the project was high, greater than 98 and 80%, in the two groups, respectively. A major problem of screening in early pregnancy is the time-lag between sampling and the availability of a test result. On average, we were able to test both partners for CF heterozygosity in 11 days.

It is extremely important that screening be voluntary. In our study very few women (2%) felt they were forced to accept the scre[Iannuzzi] although some (25%) felt it difficult to refuse an offered test. Information about their CF carrier status shocked or worried the majority of the women and, to a lesser extent, their partners. However, most were helped by genetic counselling [unpubl. results].

With a detection rate of carriers of 90%, we can identify 81% of all couples at risk for having a child with CF. If, on average, 82% of women offered screening accept the test, then with an annual number of births in Denmark of 60,000 we will have to test 49,200 women plus 1,273 partners in order to identify 34 pregnancies with a 1-in-4 risk. If all couples opt for prenatal diagnosis, 9 CF fetuses will be found. The cost per test including counselling will be approximately 300 Danish crowns (£30), i.e., a total sum of approximately 18 million crowns, including the price of prenatal diagnosis and abortion. The average annual cost of t[Kerem]ent of a CF child is 500,000 crowns [The Danish CF Centre, pers. commun]. With an average life span of 30 years this will amount to 15 million crowns per CF patient. Although these are a very rough estimates of costs, financial considerations certainly favour the proposed screening programme for CF.

From the data obtained, a true carrier frequency for CF can be calculated. We found (172 + 3) carriers among (6,599 + 162) persons tested giving a carrier frequency of 1:38.6. With a detection rate of 88%, the true carrier frequency will be 1:34.5. This gives a true incidence of CF of 1:4,761, which is remarkably close to the previous estimate of 1:4,700 [8], suggesting that most, if not all, Danish CF patients were identified in the period of 1945 to 1985.

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