MUTATION ANALYSIS FOR HETEROZYGOTE DETECTION AND THE PRENATAL DIAGNOSIS OF CYSTIC FIBROSIS

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Abstract The cystic fibrosis gene was recently cloned, and a three-base deletion removing phenylalanine 508 from the coding region was identified as the mutation on the majority of cystic fibrosis chromosomes. We used the polymerase chain reaction and hybridization with allele-specific oligonucleotides to analyze the presence or absence of this mutation on 439 cystic fibrosis chromosomes and 433 normal chromosomes from non-Ashkenazic white families.

This mutation was present on 75.8 percent of the cystic fibrosis chromosomes. Using the DNA markers XV-2c and KM-19, we found that 96 percent of cystic fibrosis chromosomes with the mutation had a single DNA haplotype that occurs frequently with cystic fibrosis chromosomes. This haplotype was also found on 54 percent of the cystic fibrosis chromosomes without the three-base deletion. The three-base deletion was found on only 30.3 percent of cystic fibrosis chromosomes from Ashkenazic families, although the common cystic fibrosis haplotype was present on 97 percent of cystic fibrosis chromosomes from Ashkenazic families.

The ability to detect the common mutation causing cystic fibrosis represents a major improvement in prenatal diagnosis and heterozygote detection, particularly in families in which no DNA sample is available from the affected child, and provides an improved method of testing for spouses of carriers of cystic fibrosis. Mutation analysis introduces the possibility of population-based screening programs for carriers, which on the basis of the sample in this study, would currently identify about 57 percent of the non-Ashkenazic white couples at risk. (N Engl J Med 1990; 322:291-6.)

CYSTIC fibrosis is the most common severe autosomal recessive genetic disorder affecting the white population. A review of the clinical, physiologic, and genetic aspects of cystic fibrosis has been published recently.[1] The reported disease frequency in white populations varies markedly, but the incidence in North American whites is probably close to 1 in 2500.[1] This implies a gene frequency of 1 in 50 and a carrier frequency of about 1 in 25.5. The cystic fibrosis locus was linked to a DNA polymorphism in 1985,[2] and the gene was localized to the long arm of human chromosome 7.[3-5] Subsequently, segments of DNA progressively closer to the cystic fibrosis gene were identified, and DNA polymorphisms with particular alleles frequently associated with the cystic fibrosis gene (i.e., linkage disequilibrium) were found.[6] The linkage-disequilibrium data implied that the cystic fibrosis chromosomes in a large proportion of cases arose from one or a few mutational events. The cloning of the cystic fibrosis gene has been reported,[7-9] and a three-base deletion (designated ΔF508) that removes phenylalanine 508 from the 1480–amino acid coding region was identified as the mutation that causes cystic fibrosis in the majority of cases. The sequence of the predicted product of the cystic fibrosis gene suggests that it is a membrane-associated protein with ATP-binding sites.[8]

Genetic counseling for cystic fibrosis began with standard risk calculations. In the mid-1980s, the demonstration of decreased levels of fetal microvillar intestinal enzymes in amniotic fluid from women carrying fetuses with cystic fibrosis provided a good but not absolute method of prenatal diagnosis.[10,11] With the availability of tightly linked DNA markers, prenatal diagnosis and carrier detection based on linkage analysis became possible.[12-15] Because chromosomes with certain haplotypes are more likely to carry a cystic fibrosis mutation, linkage-disequilibrium data can be combined with linkage analysis and the analysis of microvillar intestinal enzymes for genetic counseling and risk calculation.[15-17] The availability of direct detection of mutations causing cystic fibrosis marks a new era for genetic counseling and prevention. In this study, we report the results of mutation analysis in more than 200 families who have undergone DNA analysis for cystic fibrosis. We have examined the effect on prenatal diagnosis and heterozygote detection within families, and we review some implications for population-based heterozygote screening.

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METHODS

Subject Population

Mutation analysis was performed in Houston on a large series of families in which members have cystic fibrosis. Families were referred from throughout North America, beginning in 1985. They were considered white if they were of European ethnic origin; these included a small number of families of southern European origin (Italian, Spanish, Greek, and Portuguese). Ashkenazic and Hispanic families were analyzed separately; a few Ashkenazic subjects may have been included in the analysis of white families because of a lack of background information. Hispanic parents of children with cystic fibrosis who reported recent full European ancestry were in-

cluded in the analysis of whites, whereas others of presumed mixed Native American and European ancestry were counted as Hispanic. North American black, Asian, and Middle Eastern families were excluded from the analyses. The parents and the affected child were studied only when the diagnosis of cystic fibrosis met standard criteria.[1]

DNA Analysis

Restriction-fragment–length polymorphisms were determined for the XV-2c and KM-19 probes as described previously, and haplotypes were constructed.[15] Data are also shown for a *PstI* polymorphism at the D7S8 locus,[18] designated JGP in Figure 2. The absence of a restriction-enzyme site for each polymorphism is indicated as the 1 allele and the presence of the site as the 2 allele. The haplotypes for (XV-2c)-(KM-19) are as follows: A is 1-1, B is 1-2, C is 2-1, and D is 2-2.[15]

Genomic DNA from whole blood containing EDTA as an anticoagulant was prepared by mixing it with an equal volume of lysis buffer (0.32 M sucrose, 10 mM TRIS–hydrochloric acid [pH 7.5], 5 mM magnesium chloride, and 1 percent Triton X-100). The nuclear leukocyte pellet was washed in the lysis buffer and then processed for DNA preparation. Leukocyte pellets, direct chorionic-villus samples, and cultured cell pellets were suspended in ABI proteinase K solution (Applied Biosystems, Foster City, Calif.) and then processed either manually or with the ABI nucleic acid extractor for phenol extraction and ethanol precipitation with the use of the ABI reagents and protocol. DNA was subjected to amplification as recommended by the manufacturer of the polymerase (Perkin–Elmer Cetus, Emeryville, Calif.) except that a lower concentration of *Taq* polymerase was used. Each 50-μl reaction mixture contained 50 mM potassium chloride, 10 mM TRIS–hydrochloric acid (pH 7.8), 1.5 mM magnesium chloride, 0.01 percent (wt/vol) gelatin, 200 μM of each deoxynucleotide triphosphate, 1.0 μM of each oligonucleotide primer, 1 μg of genomic DNA, and 1.25 units of *Taq* polymerase. Two drops of mineral oil were added to each reaction.

The amplification sequences were 5′GTTGGCATGCTTTGATGACGCTTC-3′ and 5′GTTTTCCTGGATTATGCCTGGGCAC-3′.[9] The correct sequence for the second primer should be 5′GTTTTCCTGGATTATGCCTGGCAC-3′.[9] The amplification of DNA was performed for 25 or 30 cycles; each cycle consisted of 1 minute of denaturation at 94°C, 30 seconds of annealing at 53°C, and 2 minutes of polymerization at 72°C. After amplification, 350 μl of a solution consisting of 0.4 N sodium hydroxide, 25 mM EDTA, and 0.01 percent bromophenol blue was added to the microcentrifuge tube. Each tube was then mixed by vortexing and centrifuged to bring the mineral oil to the surface. Aliquots of 100 μl were applied to a Schleicher and Schuell Minifold II slot–blot apparatus (Keene, N.H.) (care was taken to avoid the delivery of any mineral oil to the well) and filtered by vacuum according to the instructions of the manufacturer. The DNA was bound to a Zeta-Probe nylon membrane (Bio-Rad Laboratories, Richmond, Calif.) that was first soaked momentarily in boiling deionized water and then soaked in 2× standard saline citrate until use (1× standard saline citrate is 0.15 M sodium chloride and 15 mM sodium citrate, pH 7.0). After removal from the slot–blot apparatus, the filters were baked for two hours at 80°C in a vacuum drying oven. Prehybridization was performed in 0.5 M sodium phosphate (pH 7.2) and 7 percent sodium dodecyl sulfate at 55°C for at least 30 minutes.

The allele-specific oligonucleotide for the detection of the normal sequence was 5′CACCAAAGATGATATTTTC-3′, and the sequence for the allele-specific detection of the mutation was 5′-AACACCAATGATATTTTCTT-3′.[9] Allele-specific probes were prepared by labeling the 5′ end with γ[^{32}P]ATP and polynucleotide kinase,[19] followed by purification with NENSORB-20 cartridges (Dupont, Wilmington, Del.) according to the manufacturer's instructions. Approximately 2 to 3×10^6 Cherenkov counts of the probe was added to 8 ml of hybridization solution for a single filter from the slot–blot apparatus; the amounts were doubled for two or three filters. Hybridization was performed in prehybridization buffer with the addition of 25 μg of sonicated, boiled salmon sperm DNA per milliliter at 55°C for at least four hours but usually overnight. Filters were washed in 500 ml of 40 mM sodium phosphate (pH 7.2) and 1 percent sodium dodecyl sulfate for 5 minutes at room temperature, followed by a second wash in 500 ml for 15 minutes at 40°C. Filters were exposed with Kodak XAR-5 film (Rochester, N.Y.) with an intensifying screen for six to eight hours.

Risk Calculations

Risks were calculated from the Hardy–Weinberg equilibrium of $p^2 + 2pq + q^2 = 1$, where p is the frequency for a normal allele at the cystic fibrosis locus (0.98), q is the frequency for a disease allele (0.02), p^2 is the frequency of noncarriers, 2pq is the frequency of carriers, and q^2 is the frequency of patients with cystic fibrosis. If d is the rate of detection of cystic fibrosis mutations, then 2pqd is the frequency of carriers with a positive mutation test and $2pq(1 - d)$ is the frequency of carriers with a negative mutation test. Alternatively, these probabilities can be calculated by Bayesian analysis. The corrected carrier risk (the risk that a person with a negative test is a carrier) is the frequency of carriers with a negative test divided by the sum of that frequency and the frequency of noncarriers:

$$\text{Corrected carrier risk} = \frac{2pq(1 - d)}{2pq(1 - d) + p^2}.$$

If one parent has a positive mutation test and the other a negative test, the risk of cystic fibrosis in the offspring is ¼ × the corrected carrier risk. If both parents have a negative mutation test, the risk of cystic fibrosis in the offspring is ¼ × (corrected carrier risk)^2.

In the context of population-based carrier testing, the rate of detection of couples at risk is equal to d^2, the rate of detection of one partner for couples at risk is $2d(1 - d)$, and the rate of failure to identify either partner for couples at risk is $(1 - d)^2$.

Results

Typical results for the direct analysis of the three-base deletion with amplification of genomic DNA and hybridization with allele-specific oligonucleotides are shown in Figure 1. The DNA from the affected child shows hybridization only with the mutant allele-specific oligonucleotide (homozygous for the mutation), whereas the DNA from the parents shows hybridization with both the mutant and the normal allele-specific oligonucleotides (heterozygous for the mutation). Both chromosomes carrying the cystic fibrosis gene in this family had the typical B haplotype for XV-2c and KM-19 (see Methods for details of the haplotypes), whereas both normal chromosomes had the C haplotype. The genotypes for carrier and noncarrier siblings, which were based previously on linkage analysis, were confirmed by the analysis with allele-specific oligonucleotides.

The presence or absence of the three-base deletion was determined and correlated with haplotypes for XV-2c and KM-19 for 439 chromosomes carrying a mutation for cystic fibrosis and 433 normal chromosomes by analysis of affected children and their parents from non-Ashkenazic, white families, as shown in Table 1. Each affected child has two cystic fibrosis chromosomes, one derived from each parent, and each parent has one normal chromosome. The data confirm that the three-base deletion is found in the majority (75.8 percent) of cystic fibrosis chromosomes in the white population. This mutation was found almost exclusively (96 percent) on chromosomes bearing the B haplotype, which was previously shown to be highly associated with cystic fibrosis chromosomes.[15,20,21] However, the three-base deletion was not found on all cystic fibrosis chromosomes carrying the B haplo-

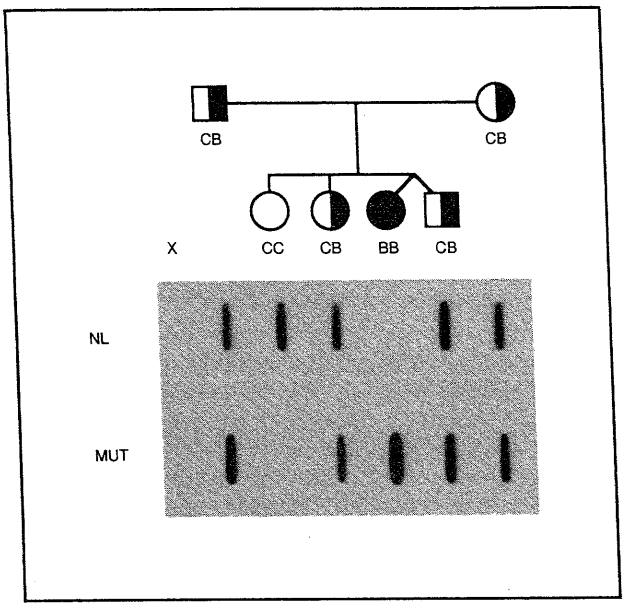

Figure 1. Detection of the Three-Base Deletion Causing Cystic Fibrosis with the Use of DNA Amplification and Allele-Specific Oligonucleotides.

Squares denote male family members, and circles female members. Solid symbols denote affected members, half-solid symbols heterozygotes, and open symbols noncarriers. NL denotes the autoradiogram with the normal allele-specific oligonucleotide, and MUT the autoradiogram with the mutant allele-specific oligonucleotide. The X denotes a blank lane in which no genomic DNA was added to the polymerase chain reaction. Letters below the pedigree symbols indicate the XV-2c and KM-19 haplotypes as described in Methods.

type and was occasionally seen on cystic fibrosis chromosomes bearing other haplotypes, as observed in the initial report of this mutation.[9] The B haplotype was still the most common haplotype seen with cystic fibrosis chromosomes not bearing the three-base deletion, suggesting that a moderately common second mutation may exist on chromosomes with this haplotype.

On the basis of the results shown in Table 1, the predicted frequencies of patients with cystic fibrosis homozygous for this mutation, heterozygous for this mutation, and homozygous for the absence of this mutation are 57.4, 36.7, and 5.9 percent, respectively, whereas the observed frequencies were 58.2, 33.7, and 8.0 percent. There may be a minor deficiency of heterozygotes due to preferential mating within ethnic groups in which the mutation is less frequent (perhaps the southern European families[20,21] or rare unidentified Ashkenazic families in the group; see below). However, the cystic fibrosis chromosomes with and without this mutation were quite well distributed in this North American population.

Table 1 does not include the normal chromosome for three parents (two fathers and one mother) of children with cystic fibrosis who initially appeared to be homozygous for the three-base deletion. Amplification of these three samples with other oligonucleotide primers from the flanking introns rather than from within the exon yielded the same pattern of results when analyzed with the allele-specific oligonucleotides. Further analysis revealed that these three persons were compound heterozygotes, with one chromosome carrying the three-base deletion and the other chromosome carrying either of two missense mutations within the region of the allele-specific oligonucleotide (Kobayashi K, et al.: unpublished data). Detailed clinical analysis of these three persons was normal. The three chromosomes carrying missense mutations were found among 330 chromosomes for which the analysis would have detected the phenomenon.

Data for chromosomes from Ashkenazic and Hispanic families are summarized in Table 2. The three-base deletion was much less common in the Ashkenazic population, being present on only 30.3 percent of cystic fibrosis chromosomes as compared with 75.8 percent in other whites (chi-square = 29.8; $P<0.001$). The linkage disequilibrium was extremely high in the Ashkenazic population, with 97 percent of cystic fibrosis chromosomes carrying the B haplotype, whereas the B haplotype was quite rare on normal chromosomes. The data suggest that a second common mutation occurs with the B haplotype in the Ashkenazic population and is yet to be identified. The data for chromosomes from the Hispanic population were very similar to those for chromosomes from whites, with 12 of 17 cystic fibrosis chromosomes (71 percent) having the three-base deletion; this suggests that cystic fibrosis occurs in the Hispanic population primarily through European admixture.

Direct analysis for the three-base deletion has been applied in numerous new case studies and in families studied previously for heterozygote detection and prenatal diagnosis, as shown in Figure 2. Case 1 represents a family that is fully informative for prenatal diagnosis according to both linkage and mutation analysis, and such analyses indicate that the fetus is affected; the family is continuing the pregnancy. Case 2 was uninformative for one parent according to linkage analysis, and the other parent contributed the cystic fibrosis chromosome to the fetus, as demonstrated by a polymorphism at the D7S8 locus (JGP). The

Table 1. Mutation Analysis of Cystic Fibrosis and Normal Chromosomes.

Haplotype	Cystic Fibrosis Chromosomes				Normal Chromosomes
	with 3-base deletion		without 3-base deletion		
A	1		20		136
B	314		57		62
C	0		13		163
D	11		9		60
Haplotype phase not clear	7*		7*		12
Total	333	+	106	= 439	433

*It is highly probable that the seven chromosomes with the mutation consist of six with the B haplotype and one with the D haplotype and that the seven chromosomes without the mutation represent six with the C haplotype and one with the D haplotype.

Table 2. Mutation Analysis of Chromosomes from Ashkenazic and Hispanic Subjects.

HAPLOTYPE	ETHNIC ORIGIN	CYSTIC FIBROSIS CHROMOSOMES		NORMAL CHROMOSOMES
		WITH 3-BASE DELETION	WITHOUT 3-BASE DELETION	
B	Ashkenazic	9	23	2
Non-B	Ashkenazic	1	0	31
B	Hispanic	10	1	3
Non-B	Hispanic	1	2	11
No data	Hispanic	1	2	3

fetus had a 50 percent risk of cystic fibrosis according to linkage analysis, but this risk was decreased by the results of intestinal-enzyme analysis, which were normal. According to mutation analysis, the fetus is a carrier.

Case 3 depicts a family in which there was no DNA sample available from the deceased affected child, but linkage-disequilibrium data predicted that the fetus is not a carrier, since the cystic fibrosis mutation is probably on the chromosome with the B haplotype in both parents. The results of intestinal-enzyme analysis were normal, and mutation analysis confirmed that the fetus is not a carrier. Case 4 depicts a family in which no DNA was available from the deceased affected child, but linkage-disequilibrium data predicted that the fetus probably was a heterozygote. The results of fetal intestinal-enzyme analysis were abnormal, and a Bayesian calculation yielded a 50 percent risk of cystic fibrosis for the fetus. The pregnancy was terminated, but retrospective mutation analysis indicated that the fetus was not affected with cystic fibrosis. This termination of pregnancy could have been avoided if mutation analysis had been available. Case 5 depicts a maternal crossover in which the XV-2c marker predicted that the fetus was affected with cystic fibrosis, whereas the D7S8 marker (JGP) predicted that the fetus was a carrier for cystic fibrosis; XV-2c and D7S8 are known to flank the cystic fibrosis locus. The results of intestinal-enzyme analysis were normal, and the family chose to continue the pregnancy. The child has been born, and the results of the sweat test were normal. The mutation analysis indicated a heterozygote child, and the result is consistent with the interpretation that the three-base deletion is indeed the mutation causing cystic fibrosis in this family. Case 6 depicts carrier testing in a number of relatives of a child with cystic fibrosis. Aside from the parents of the affected child, only one of the six family members tested was found to be a carrier.

The risks of being a carrier among persons without a family history of cystic fibrosis and the risks of cystic fibrosis among the offspring of couples undergoing mutation analysis are shown in Table 3. (A table of risks of being a carrier among persons with various XV-2c and KM-19 genotypes and a negative muta-

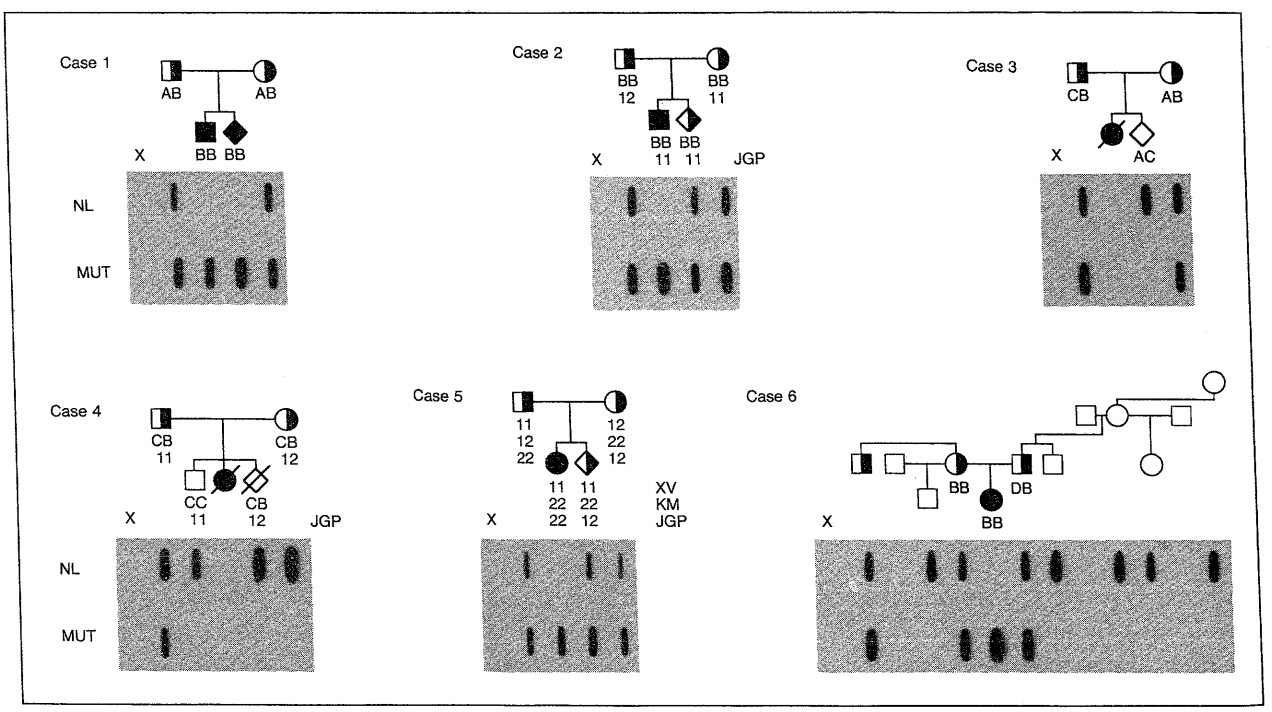

Figure 2. Mutation Analysis in Families with a History of Cystic Fibrosis for Prenatal Diagnosis and Heterozygote Detection.
Pedigree symbols are as in Figure 1, except that diamonds denote fetal samples, and open symbols noncarriers or members whose genotype was uncertain. Slashes denote deceased family members. Haplotypes are presented as in Figure 1, but the allele data are presented for XV-2c, KM-19, and a polymorphism at the D7S8 locus (JGP) as numbers below the pedigree symbols. Samples with no DNA added to the polymerase chain reaction are marked by an X, and other lanes in which there is no signal for either allele-specific oligonucleotide are instances in which no DNA sample was available from the family member in the pedigree.

Table 3. Cystic Fibrosis–Related Risks after Mutation Analysis for Carrier Status.

% of Cystic Fibrosis Mutations Detectable	Carrier Risk for Person with Negative Test	Risk of Cystic Fibrosis in Offspring for Couples Tested	
		one parent positive*	neither parent positive
0	1 in 25.5	NA	1 in 2,500
70	1 in 82.7	1 in 331	1 in 27,400
75	1 in 99.0	1 in 396	1 in 39,200
80	1 in 124	1 in 494	1 in 61,000
85	1 in 165	1 in 661	1 in 109,200
90	1 in 246	1 in 984	1 in 242,100
95	1 in 491	1 in 1964	1 in 964,400

*NA denotes not applicable.

tion analysis is available from the authors.) The effectiveness of the detection of couples at risk on the basis of population-based carrier testing is shown in Table 4.

Discussion

Previous work indicated that the predominant mutation causing cystic fibrosis in the white population is a three-base deletion that is easily detected by direct analysis,[9] and this conclusion is confirmed by the current study. The presence of the mutation on 68 percent of cystic fibrosis chromosomes in the original study,[9] as compared with 76 percent in this report, can be explained by ethnic differences in the subjects studied and by the separate analysis of the cystic fibrosis chromosomes from Ashkenazic families in this report. The simple two-allele haplotypes (XV-2c and KM-19) used in this study are approximately 200 kb centromeric to the three-base deletion. In comparison with the previous study,[9] 38 percent of the chromosomes with the D haplotype described here may correspond to the common, extended haplotype Ia, and 35 percent of those with the B haplotype (the common cystic fibrosis haplotype here) may correspond to haplotypes other than Ia.

For the purposes of the remainder of the discussion, it seems reasonable to assume that additional mutations accounting for various proportions of the remaining cystic fibrosis chromosomes will be identified relatively soon and will be detectable by direct analysis. Mutation analysis has an immediate major effect on programs offering genetic counseling, heterozygote detection, and prenatal diagnosis for families with a history of cystic fibrosis. With the use of this method, a majority of families were either fully informative (58.2 percent) or partially informative (33.7 percent). Numerous families seek heterozygote detection or prenatal diagnosis after an affected family member has died, when no DNA sample is available from that member. Mutation analysis offers a substantial improvement in the ability to predict the likelihood of cystic fibrosis in the offspring of the majority of these families. In addition, mutation analysis is helpful in difficult clinical circumstances, such as a course suggestive of cystic fibrosis in a deceased newborn, the presence of echogenic masses suggestive of meconium ileus on prenatal ultrasound, or the difficulty of diagnosis in a patient with borderline sweat-test results.

Genetic counseling for close relatives of patients with cystic fibrosis that uses linkage analysis, linkage disequilibrium, and microvillar-intestinal-enzyme analysis has been reviewed recently.[15] Previous diagnostic strategies have been altered because mutation analysis improves the detection of carriers among the relatives of patients with cystic fibrosis, but more important, because mutation analysis can be applied to the spouses of these relatives. Until now, DNA analysis for carrier testing in the relatives of patients with cystic fibrosis was often not encouraged because of cost considerations and because a definitive diagnosis of cystic fibrosis in a fetus was virtually impossible except for pregnancies at a one-in-four risk. With the availability of mutation analysis, such testing can now be encouraged. If one parent has a close relative with cystic fibrosis and is found to be a carrier on the basis of mutation or linkage analysis, we believe that mutation analysis of the spouse is indicated. If both parents are definite carriers, there is a one-in-four risk that the child will be affected, and prenatal diagnosis is possible. If the low-risk spouse has a normal result on mutation analysis, further studies are complex and probably not useful in most cases. If the parent who has a close relative with cystic fibrosis is a carrier, the carrier risk for the spouse is reduced to 1 chance in 99 by a negative test (with the use of 75 percent mutation detection; Table 3), giving a risk of 1 in 396 that their child will have cystic fibrosis. Even if the carrier parent contributes the cystic fibrosis gene to the fetus, the risk of cystic fibrosis in the fetus is still only 1 chance in 198. If the results of microvillar-intestinal-enzyme analysis are abnormal, on the basis of our estimates of false positive (0.02) and false negative (0.08) rates for this test, a Bayesian calculation yields a 19 percent probability that such a fetus would be affected with cystic fibrosis and an 81 percent probability that the intestinal-enzyme analysis would represent a false positive result. These risks could be modified further with the use of linkage-disequilibrium data, which would most often lower the risk but could increase the risk of cystic fibrosis to as high as 55 percent. Link-

Table 4. Potential for the Detection of Couples at Risk with the Use of Population-Based Screening for Carriers.

% of Cystic Fibrosis Mutations Detectable	Detection of Carrier Status		
	both parents	one parent	neither parent
		percent	
70	49.0	42.0	9.0
75	56.3	37.5	6.2
80	64.0	32.0	4.0
85	72.3	25.5	2.2
90	81.0	18.0	1.0
95	90.3	9.5	0.2

age data, linkage-disequilibrium data, and mutation analysis can be combined for quantitative risk analysis with the MLINK program from the LINKAGE package.[22]

The ability to detect cystic fibrosis mutations directly makes it feasible to consider the long-awaited possibility of population-based heterozygote screening. This is complicated by the fact that only a proportion of the mutant chromosomes can be detected at the present time. As shown in Table 4, more than half the couples at risk would be detected with the present efficiency of mutation analysis, and 90 percent of the couples at risk would be detected if mutation analysis were 95 percent effective. However, at present, the mutation can be identified in one parent but not the other or in neither parent in a large proportion of the couples at risk. Table 3 shows the risk of cystic fibrosis in the offspring of such couples. In general, if one parent is proved to carry a cystic fibrosis mutation, the risk of cystic fibrosis in the fetus is higher than the risk for the general population, but this risk decreases as the efficiency with which mutations can be detected increases. The risk of an affected fetus is substantially reduced if neither parent has a known mutation. If a mutation is identified in one of the parents, it would be possible to determine whether this parent will contribute the mutation to the fetus. However, this information is of limited value even when combined with microvillar-intestinal-enzyme analysis, because an abnormal result on microvillar-intestinal-enzyme testing is usually more likely to represent a false positive result than to represent an affected fetus, as already discussed in the context of risks within a family with cystic fibrosis.

Should population-based heterozygote screening begin immediately? On the positive side, there would be the substantial benefit of detecting approximately 57 percent of the couples at risk; only 9 percent of the Ashkenazic couples at risk would be detected. In theory, the specificity of mutation analysis might be 1.0; that is, the false positive rate should be essentially zero. In practice, genetic variations at the sites of polymerase-chain-reaction primers[23] or at the allele-specific oligonucleotide site might cause false positive or false negative results (as found in three subjects in our series). Some delay may be appropriate, since additional mutations will probably be identified soon, allowing a more effective carrier-detection program. Although additional mutations should be identified quickly, a large number of mutations may account for the last few percent of cystic fibrosis chromosomes. It seems appropriate at this time to initiate pilot programs for population-based heterozygote screening, with the expectation that such programs will become widespread very rapidly. A recent survey of the attitudes of geneticists from 18 nations indicated considerable differences about the preferred age for screening and about whether screening should be required or by consent only.[24] The option for carrier screening for cystic fibrosis will probably become the standard of care in many countries in the future. Health care providers should plan for this development. Population-based heterozygote screening for cystic fibrosis would require education programs for the general population, and the general level of genetic knowledge and awareness would increase substantially as a result.

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