CLINICAL VALIDITY

Question 18: How often is the test positive when the disorder is present?
Question 19: How often is the test negative when the disorder is not present?
Question 20: Are there methods to resolve clinical false positive results in a timely manner?
Question 21: What is the prevalence of the disorder in this setting?
Question 22: Has the test been adequately validated on all populations to which it may be offered?
Question 23: What are the positive and negative predictive values?
Question 24: What are the genotype/phenotype relationships?
Question 25: What are the genetic, environmental or other modifiers?

CLINICAL VALIDITY

Question 18: How often is the test positive when the disorder is present?
Question 19: How often is the test negative when the disorder is not present?

Summary

- Clinical sensitivity is dependent on the mutation panel used and the racial/ethnic makeup of the population to be tested
- Clinical sensitivity is not dependent on the screening model used.
- Using the recommended panel of 25 mutations, the clinical sensitivity (proportion of carrier couples or affected fetuses correctly classified) is:
  - 78 percent among non-Hispanic Caucasian couples
  - 52 percent among Hispanic Caucasian couples
  - 42 percent among African American couples
  - 88 percent among Ashkenazi Jewish couples
  - 24 percent among Asian American couples
- Actual clinical sensitivity is likely to be slightly lower because analytic sensitivity is less than 100 percent (estimated in Question 9 to be 97.9 percent).

- Based on proficiency testing results alone, an analytic false positive result will likely occur about 5 times in every 1000 tests.
- The clinical specificity cannot be reliably estimated, because the impact of confirmatory testing is undocumented.
- Assuming routine confirmatory testing of all positive test results, false positive couples results are likely to be corrected, but the extent of correction is unknown.

- The 'initial positive rate' (proportion of couples/partners receiving positive test results) varies by model used for screening, as well as by factors relating to race/ethnicity and panel composition. Using the recommended panel of 25 mutations among non-Hispanic Caucasian couples, the initial positive rate is
  - 3.4 percent for the sequential model (percent of identifiable mutations in screened women)
  - 0.1 percent for the couple model (percent of identifiable mutations in both partners)
  - 6.7 percent for the concurrent model (percent of identifiable mutations in either of the screened partners)

Introduction

The definitions of clinical sensitivity (Question 18) and clinical specificity (Question 19) can be derived using a two-by-two contingency table for data from either case/control or cohort studies. If the data are from a general population cohort, both positive predictive and negative predictive values (Question 23) can also be directly computed. Table 3-1 shows the definitions for these four screening characteristics, assuming that a general population of pregnancies is being tested.

The rows are defined by the test results. In this instance, the DNA screening test for cystic fibrosis is considered positive when both partners of a couple have an identifiable mutation, regardless of the screening model. The first row includes all screen positive couples, and the second row includes all couples who are not screen positive. The columns are defined by what the screening test aims to detect. In this instance, it is to identify carrier couples who are at a 1 in 4 risk (1:3 odds) for having a child with cystic fibrosis. The first column indicates couples who are both carriers of a cystic fibrosis mutation, and the second column indicates couples who are not.

Table 3-1. A Two-by-Two Contingency Table for Deriving the Four Major Clinical Performance Parameters

	Both Partners are Cystic Fibrosis Carriers		
	Yes	**No**	**Totals**
Couple Positive by DNA Testing			
Yes	A	B	A+B
No	C	D	C+D
Totals	A+C	B+D	A+B+C+D

- Clinical sensitivity [A / (A + C)] is the proportion of couples in which both partners are cystic fibrosis carriers (A+C) and who are correctly identified as being positive (A) by the screening test.
- Clinical specificity [D / (B + D)] is the proportion of non-carrier couples (B+D) who are correctly identified as being negative (D) by the screening test.
- Positive predictive value [A / (A + B)] is the proportion of positive tests (A + B) that correctly identifies carrier couples (A).
- Negative predictive value [D / (C + D)] is the proportion of negative tests (C + D) that correctly identifies non carrier couples (D).

Figure 1 shows an example of applying prenatal screening for cystic fibrosis to a hypothetical cohort of 1,000,000 couples. In this example, the prevalence of cystic fibrosis is 1:2,500 (carrier rate 1/25), and the DNA test panel identifies 77 percent of the carrier couples (if 88 percent of mutations are detectable in each individual, then 88 percent squared, or 77 percent are detectable in the couple). The analytic sensitivity is taken to be 97.9 percent (Question 9), and the analytic specificity (after confirmatory testing) is assumed to be, in this example, 99.99 percent (false positive rate of 1 per 10,000 individuals tested). Among the population screened, there are 1,600 carrier couples ($1{,}000{,}000 * (1/25)^2$). 77 percent of the 1,600 carrier couples are detectable (1,232), and 1,181 of these are detected ($1{,}232 * .979^2$). Among the 998,400 non-carrier couples, 76,800 will include one carrier partner, and, in six of these couples, a false positive result will occur in the non-carrier partner (76,800*.88*.979*0.0001). The numbers from Figure 3-1 can now be entered into a two-by-two table (Table 3-2) by substituting actual numbers into the format shown earlier in Table 3-1. The clinical performance estimates can then be computed.

Figure 3-1. A Schematic Showing the Results of Prenatal Cystic Fibrosis Screening for 'Carrier Couples'

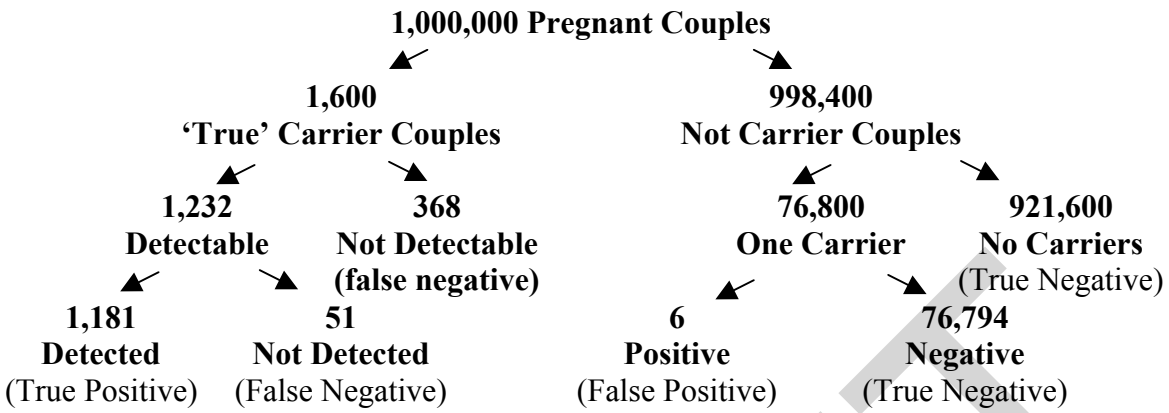

Table 3-2. A Two-by-Two Contingency Table for Deriving the Four Major Clinical Performance Parameters in a Hypothetical Population of 1,000,000 Couples

	Both Partners are Cystic Fibrosis Carriers		
	Yes	**No**	**Totals**
Couple Positive by DNA Testing			
Yes	1,181	6	1,187
No	419	998,394	998,813
Totals	1,600	998,400	1,000,000

Clinical sensitivity = 74 percent (1,181/1,600 * 100)
Clinical specificity = 99.9994 percent (998,394/998,400 * 100)
Positive predictive value = 99.5 percent (1,181/1,187 * 100) odds 196:1
Negative predictive value = 99.96 percent (998,394/998,813 * 100) odds 2355:1

Impact of the screening model on these estimates

In Figure 3-1, there are a total of 76,800 couples with one partner a carrier, and all are considered as having positive test results. Only the expanded one-step (concurrent) screening model will identify all of these carriers. In that model, samples are obtained and tested from both partners. The other two screening models (the two-step or sequential, and the one-step or couple) only identify half of the carrier/non-carrier couples, thereby reducing the number of clinical false positive results from six to three couples. The following sections consider additional issues relating to clinical sensitivity and specificity.

Clinical sensitivity

Clinical sensitivity refers to the proportion of carrier couples (or affected fetuses) that can be detected by screening couples during pregnancy. In contrast, analytic sensitivity describes how often the laboratory correctly identifies a mutation that is included in its panel. Because of the large number of mutations responsible for cystic fibrosis and the limited number of mutations that can currently be economically included in a prenatal screening setting, not all carrier individuals will be identified. Thus, clinical sensitivity can be relatively low, even when analytic sensitivity is relatively high. Table 3-3 shows the clinical sensitivity as a function of the proportion of mutations detected (assuming that the analytic sensitivity is 100 percent). For example, the table shows that, under the assumption of Hardy-Weinberg, it is necessary to identify 70 percent of the mutations in order to detect 49 percent of the carrier couples. In this example, the clinical sensitivity is 49 percent.

Table 3-3. Clinical Sensitivity: Proportion of Carrier Couples (or Affected Fetuses) Identified as a Function of the Proportion of Cystic Fibrosis Mutations Detected by a Given Screening Panel

Proportion of Mutations Detected (%)	Clinical Sensitivity (%)
20	4
30	9
40	16
50	25
60	36
70	49
75	56
80	64
85	72
90	81
95	90

Individual mutation frequencies in the non-Hispanic Caucasian population

Because of the way that race/ethnicity is collected, it is not always possible to define race/ethnicity in consistent and highly stratified groupings. The United States Government, for example, usually stratifies data into three racial categories (Caucasians, Blacks and Asians) and into Hispanic/non-Hispanic ethnicity. Many other data sources are also stratified in this way (e.g., Cystic Fibrosis Foundation Patient Database). The analyses presented here are, of necessity, stratified according to the methods used by those sources. When possible, more finely stratified groups are also considered (e.g., Ashkenazi Jewish).

In order to estimate the proportion of carrier couples (or affected fetuses) that can be identified for any given panel of mutations, it is necessary to obtain the mutation frequencies in an unbiased sampling of individuals clinically affected with cystic fibrosis. Table 3-4 shows the frequencies of the 25 mutations in the panel recommended for prenatal screening. The table includes two studies of non-Hispanic Caucasians with cystic fibrosis. The mutations are listed in

order of decreasing average frequency. As an indicator of reliability of these mutation frequencies, bolded entries indicate that the mutation was tested by more than one-quarter of the laboratories (CF Consortium) and was observed more than 10 times (CF Consortium and CF Foundation).

Table 3-4. Mutation Frequencies for non-Hispanic Caucasians in the United States Within the Recommended 25 Mutation Panel

		Mutation Frequency (%)			
Num	**Mutation**	**CF Consortium [1]**	**CF Foundation [2]**	**Average (%)**	**Cumulative (%)**
1	delF508	**68.94**	**75.90**	72.42	72.42
2	G542X	**2.17**	**2.39**	2.28	74.70
3	G551D	**2.06**	**2.44**	2.25	76.95
4	621+1G>T	**1.92**	**1.22**	1.57	78.52
5	W1282X	**1.42**	**1.57**	1.50	80.02
6	N1303K	**1.32**	**1.22**	1.27	81.29
8	delI507	**0.25**	**1.50**	0.88	82.17
7	R553X	**0.97**	**0.76**	0.87	83.04
9	R117H	**0.84**	**0.56**	0.70	83.74
10	3849+10kbC>T	**0.73**	**0.43**	0.58	84.32
11	2789+5G>A	0.48	**0.48**	0.48	84.80
12	1717-1G>T	**0.54**	**0.41**	0.48	85.28
13	R347P	**0.46**	**0.43**	0.45	85.73
14	711+1G>T	**0.77**	0.08	0.43	86.16
15	R560T	**0.30**	**0.46**	0.38	86.54
16	A455E	**0.54**	0.13	0.34	86.88
17	3569delC	**0.32**	**0.36**	0.34	87.22
18	G85E	**0.38**	0.20	0.29	87.51
19	R1162X	0.09	**0.36**	0.23	87.74
20	2184delA	*0.10*	0.23	0.17	87.91
21	1898+1G>A	0.09	0.23	0.16	88.07
22	R334W	0.13	0.15	0.14	88.21
23	I148T	*0.10*	0.08	0.09	88.30
24	3120+1G>T	*0.10*	0.05	0.08	88.38
25	1078delT	0.03	0.00	0.02	88.40
	Sum	**85.05**	**91.64**	**88.40**	

[1] Cystic Fibrosis Genetic Analysis Consortium (Kazazian, 1994), based on between 2,187 and 9,792 cystic fibrosis chromosomes (Appendix A)

[2] A new analysis of the Cystic Fibrosis Foundation Patient Database, based on 3,938 chromosomes (Palomaki *et al.*, 2002, – Appendix B)

Mutation frequencies derived from the Cystic Fibrosis Genetic Analysis Consortium Report The summary estimate of 85.05 percent shown above from the Cystic Fibrosis Genetic Analysis Consortium data is somewhat higher than reported (Kazazian, 1994), because studies that reported mainly on Hispanic, Ashkenazi Jewish or African American affected individuals are removed from the present analysis. Also, the denominators for each of the mutation frequencies are computed in the present analysis by dividing the number of observed chromosomes by the total number of chromosomes reported only for studies that actually tested for the given mutation. The earlier report (Kazazian, 1994) divided the observed number of each mutation by the total number of chromosomes reported for all studies. This oversight was mentioned in the Kazazian report and has since been corrected (Giorgi *et al.*, 1997). For the more common mutations, this second correction has little or no impact. However, for the less common mutations, the corrected frequencies will be higher than originally listed. For example, the mutation frequency for A455E would be 0.28 percent prior to correction, and 0.54 percent after. The entire reanalysis is contained in Appendix A, Table 3-14. Additional information is available on-line (www.genet.sickkids.on.ca/cftr/newfreq/all.html), but it is not clear whether blank entries on these newer tables indicate "tested for and none found" or "not tested" (Markiewicz, personal communication, 2001). For this reason, they are not included. The three mutations in the recommended panel (3120+1G>T, 2814delA and I148T) that were not part of the Consortium's report have been arbitrarily assigned a frequency of 0.10 percent (italics).

There are several limitations to using these data to estimate mutation frequencies in the general population in the United States. For example, it is not possible to determine to what extent these studies included individuals who were Hispanic (or of other racial/ethnic groups). Also, some of the data reported to the consortium have been collected in reference laboratories using mutation panels of 50 or more mutations. It is possible that these expanded test panels were used selectively in cystic fibrosis patients with less common mutations that could not be identified by initial testing (e.g., the initial test may have only analyzed delF508). A major contributor to the consortium reports that this bias exists in its data (Heim *et al.*, 2001). Such a bias will lead to under-estimation of the mutation frequency for delF508. Another possible bias might be the over-representation of some racial/ethnic groups. For example, if couples of Ashkenazi Jewish heritage were to participate more fully in testing programs than other non-Hispanic Caucasian couples, this would lead to an underestimation of the delF508 mutation frequency and an over-estimation of other mutations (e.g., W1282X). The present analysis attempts to take this into account by removing any study from the analysis if its population is mainly of Ashkenazi Jewish heritage.

Mutation frequencies derived from the Cystic Fibrosis Foundation Database In an attempt to address the shortcomings of the Cystic Fibrosis Consortium data, we undertook a reanalysis of the Cystic Fibrosis Foundation Database. This data source represents approximately 85 percent of all cystic fibrosis patients in the United States. Previous analyses have been applied to the entire collection of genotypes in that database. This approach has strength in numbers (over 29,000 chromosomes studied), but it is not possible to document which mutations have been tested for by the various contributing centers. Thus, a small number of less common mutations might indicate a low mutation frequency, but it might also indicate that few patients had been tested for that mutation. This issue is addressed here by focusing only on patients attending one of nine Therapeutic Development Network (TDN) Centers that offer expanded mutation panels

to all patients. Previous analyses of the Foundation's data did not distinguish Hispanic from non-Hispanic Caucasians. The present analysis does. Of the 2,507 self-declared Caucasian individuals with cystic fibrosis attending a TDN center in 1999, 2,130 (85 percent) declared themselves to be non-Hispanic, 302 (12 percent) did not answer (or were not asked) the question, and the remaining 75 (3 percent) responded that they were of Hispanic heritage. Of the 2,130 non-Hispanic Caucasians eligible for analysis, 1,969 (92.4 percent) had been genotyped. The remaining 7.6 percent either refused genotyping, or the results were not yet available. The Foundation's data do not allow separation of Ashkenazi Jewish individuals from non-Hispanic Caucasians.

When comparing the mutation frequencies from the two sources shown in Table 3-4, the most striking difference is found in the estimate for delF508. The estimate from the Consortium is nearly 7 percentage points lower than the corrected estimate based on the Foundation's data. Based on biases that are likely to be present in the Consortium data, the Foundation's estimate may be closer to the truth. The total proportion of mutations identified from the Cystic Fibrosis Foundation Patient Database is about 6.5 percentage points higher than from the Consortium data. This overall difference is mainly due to the variation in the delF508 mutation frequency. Both estimates are higher than initially reported (Kazazian, 1994) and than generally quoted in the literature (Grody *et al.,* 2001). The following analyses use the averages of the mutation frequencies from the two studies (Table 3-4).

Table 3-5 shows the cumulative percentage of detectable mutations and the carrier couple detection rate for 1, 5, 10, 15, 20 and 25 mutations. Mutations are added in the order shown in Table 3-4 and are, therefore, only appropriate for non-Hispanic Caucasians. Mutation frequencies in other racial/ethnic groups will be considered later in this section. Figure 3-2 graphically displays the data shown in Table 3-5.

Table 3-5. A Comparison of Mutation Panel Size and Percentage of Carrier Couples Detected, Assuming an Analytic Sensitivity of 100 percent

Number of Mutations in the Panel [1]	Cumulative Percentage of Detectable Mutations	Cumulative Percentage of Carrier Couples Detected (Clinical Sensitivity)
1	72.4	52.4
5	80.0	64.0
10	84.3	71.1
15	86.5	74.8
20	87.9	77.3
25	88.4	78.1

[1] The order of added mutations is from Table 3-4

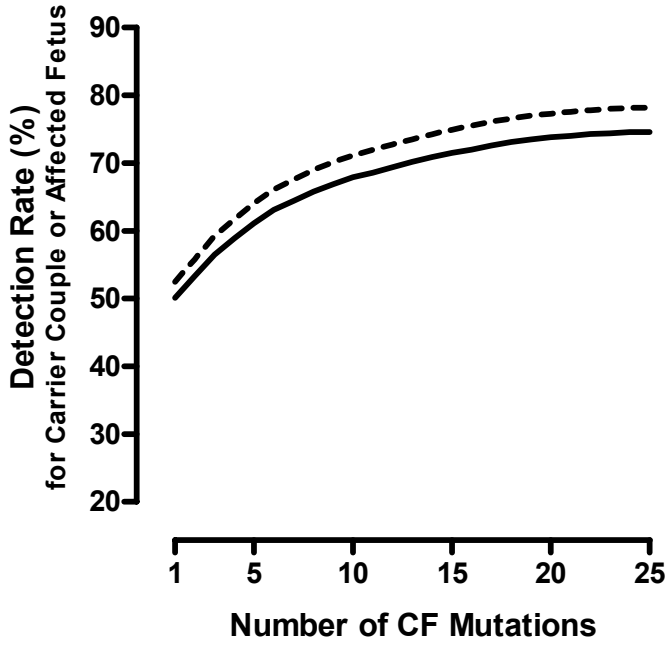

Figure 3-2. The Cumulative Percentage of Carrier Couples Detected as a Function of the Number of Mutations in the Panel. The figure is only appropriate for non-Hispanic Caucasians. The dashed line indicates the clinical sensitivity (detection rate) assuming an analytic sensitivity of 100 percent. The solid line assumes an analytic sensitivity of 97.9 percent.

Prenatal cystic fibrosis screening models and test failure rates

The impact of a test failure (i.e., no useable result) on prenatal cystic fibrosis screening depends on the model used. If a program utilizes buccal samples, the test failure rate might be, for example, 1 percent. If that program uses a two-step model, a new sample must be requested from 1 of every 100 women initially tested. Pilot trials have shown that not all individuals with sample failures will submit a second sample. If a one-step model were used instead, the couple would be considered a test failure only when both samples failed or when the second partner's sample failed when the partner had an identified mutation. Thus, the one-step model would require a repeat sample only in about 1 in 1300 couples**.** If the expanded one-step model were used, each partner would have a 1 percent chance of needing a repeat sample. Thus, about 2 of every 100 couples would need one of the partners to submit a second sample. Using blood samples would result in fewer test failures, but blood collection is associated with increased costs and may be less convenient. A summary of published pilot trials contains related information concerning failure rates and compliance with screening protocols (Question 33).

Genotype and phenotype of the fetus

The aim of prenatal screening for cystic fibrosis is two-tiered; first, to identify carrier couples and then, to offer these couples a diagnostic procedure (usually amniocentesis) and testing to identify cystic fibrosis in the fetus. When both partners are confirmed as carriers, the risk of the fetus inheriting both mutations is 1 in 4 (odds of 1:3). The relationship between genotype and phenotype will be described later in some detail (Question 24). In general, 95 percent or more of fetuses with two of the mutations contained in the screening panel will have manifestations that include serious lung problems, indicating that the cystic fibrosis mutations are of high 'penetrance'.

Clinical specificity

The analysis in this section is restricted to screened couples who, because of their genetic makeup, cannot have a child with cystic fibrosis. Rarely, these couples may be incorrectly classified as a carrier couple (i.e., a mutation is reported in both partners). Clinical specificity is a measure of how often this occurs. The following lists several types of errors that might lead to such misclassification:

pre-analytic errors

- a sample mix-up prior to receipt by the laboratory
- a degraded or mishandled sample
- non-paternity

analytic errors

- a sample mix-up after receipt by the laboratory
- benign polymorphism mistaken for mutation

post-analytic errors

- data entry error
- incorrect laboratory interpretation of assay results
- report mix-up at the laboratory or health provider site

According to the analysis shown earlier (Question 9), the analytic specificity is 97.9 percent. (i.e., a mutation is falsely reported to be present, or the wrong mutation is reported in about 2 to 3 per hundred tests). This rate is derived from external proficiency testing and, therefore, may not reflect the checks and balances routinely in place in the clinical laboratory that are designed to identify and correct analytic errors. Most of the errors identified by proficiency testing consist of assigning an incorrect mutation. Routine confirmatory procedures would likely identify and correct many of these errors (Question 14). When "wrong mutation" errors are taken into account properly, analytic specificity is increased to 99.5 percent. This analysis utilizes six years of proficiency testing data. Only one false positive result has been reported from that source for the last four years. Thus, an analytic false positive rate of 5 per 1000 cystic fibrosis mutation analyses appears reasonable. If this is true, how often will a positive couple be falsely identified? A false positive couple will occur most often in situations where one partner actually has an identified mutation. About 1 in every 28 non-Hispanic Caucasian individuals (1/25 * 0.88) will be correctly identified as being a carrier. For every thousand such couples, five of the partners might be expected to have a false positive test result. Thus, a false positive couple is expected to occur in 5 of every 28,000 non-Hispanic Caucasian couples tested (1:5600), in comparison to 35 of every 28,000 non-Hispanic Caucasian couples who are truly positive (1:800). Thus, without confirmatory testing, perhaps as many as 1 in every 7 positive couples might be incorrectly classified. How many of these are likely to be correctly reclassified by confirmatory testing? If all positive couples were to provide another sample and that sample were to be analyzed using a different methodology, perhaps all such errors would be resolved. This may not be done, however, in all screening laboratories. If such confirmatory testing is not done, these false positive couples are unlikely to be found as part of the routine diagnostic testing of the fetus, since it is expected that 3 of every 4 fetuses of true positive couples will not have two mutations present.

Some information about the possible impact of confirmatory testing is available from the European external proficiency testing program (Cuppens and Cassiman, 1995). In that program,

the protocol included a follow-up request to laboratories with incorrect cystic fibrosis testing results. They were asked to repeat the analysis. Under these unblinded conditions, three laboratories repeated the analysis of a false positive result and in all three instances, the correct genotype was confirmed. Several other false positive results were not repeated and/or not reported to the program.

References

Cuppens H, Cassiman JJ. 1995. A quality control study of CFTR mutation screening in 40 different European laboratories. *Eur J Hum Genet* **3**:235-245.

Giorgi S, Tandoi C, Ciminelli BM, Modiano G. 1997. A correction of the estimates of the least common cystic fibrosis (CF) mutations published by "The Cystic Fibrosis Genetic Analysis Consortium" in 1994. *Gene Geograph* **11**:57-59.

Grody WW, Desnick RJ. 2001. Cystic fibrosis population carrier screening: Here at last – Are we ready? *Genet Med* **3**:87-90.

Heim R, Sugarman EA, Allitto BA. 2001. Improved detection of cystic fibrosis mutations in the heterogeneous US population using an expanded, pan-ethnic mutation panel. *Genet Med* **3**:168-176.

Kazazian HH for the Cystic Fibrosis Genetic Analysis consortium. 1994. Population variation of common cystic fibrosis mutations. *Hum Mutat* **4**:167-177.

Palomaki GE, Haddow JE, Bradley LA, FitzSimmons SC. 2002. An updated assessment of cystic fibrosis mutation frequencies in non-Hispanic Caucasians. *Genet Med*, **4**:90-94.

Witt DR, Schaefer C, Hallam P, Wi S, Blumberg B, Fishbach A, Holtzman J, Kornfeld S, Lee R, Nemzer L, Palmer R. 1996. Cystic fibrosis heterozygote screening in 5,161 pregnant women. *Am J Hum Genet* **58**:823-835.

Clinical sensitivity for cystic fibrosis among Hispanic Caucasian couples

Description of the Mutation Frequency Data Table 3-6 contains the mutation frequencies for Hispanic Caucasians within the recommended panel. The two data sources are the Cystic Fibrosis Consortium and a new analysis based on the Cystic Fibrosis Foundation Patient Database. The present analysis corrects errors in the denominator of the CF Genetic Analysis Consortium data, as described for non-Hispanic Caucasians earlier in this section. The seven studies selected for analysis include individuals from the United States (4), Mexico (1) and South America (2). A listing of the data appears in Appendix C, Table 3-16. The total numbers of chromosomes tested for each mutation range from 178 to 958. Nearly half (12/25) of the 25 recommended mutations were not tested for, and these have been arbitrarily assigned a frequency of 0.1 percent.

All individuals in the Cystic Fibrosis Foundation Patient Database included in the present analysis are from the United States and have declared themselves to be both Caucasian and Hispanic. It is very likely that they were tested for at least these 25 mutations, if they were seen at a Therapeutic Diagnostic Network (TDN) Center. Only three mutations were never identified (R560T, A455E and 1898+1G>A). When the analysis is restricted to individuals from the TDN centers (the most unbiased set possible), a total of 130 chromosomes is available for analysis. With this few observations, it is possible only to obtain reliable estimates for the frequency of delF508 and the frequency of unknown/other (other mutations include those mutations that have been identified, but are not included in the recommended 25 mutations). These two frequencies are 60.0 percent (95 percent CI 51 to 68%) and 20.8 percent (95 percent CI 14 to 29%), respectively. If all individuals reporting Caucasian Hispanic ethnicity were to be included, 1,374 chromosomes are available. We have chosen to use the larger dataset, due to the increased reliability of the frequencies for less common mutations and because the overall rates for this dataset are similar to those found for patients attending the TDN Centers (Appendix B). As before, the average of the mutation frequencies from the two data sources are used as the best estimate.

As seen for non-Hispanic Caucasians, the frequency of delF508 is about 15 percent higher in the CF Foundation Patient Database than in the CF Consortium data (60 versus 45 percent). It is not clear whether this difference is due to underascertainment of delF508 in the Consortium data (as described earlier) or to some other bias that may cause an overestimate in the CF Foundation data. The 12 'rare' mutations that were not tested for in the Consortium's data collection were each assigned a frequency of 0.10 percent for a total of 1.2 percent This is somewhat lower, but consistent with, the observed rate of 2.0 percent. The cumulative frequency for the 25 mutations is about 70 percent, with estimates ranging from as low as 60 to as high as 80 percent. One possible source of bias could explain the relatively high frequency of delF508 in the CF Foundation Patient Database. That dataset relied on self-reported racial/ethnic information, and it is likely that some fraction of the population had parents or grandparents who were of Northern European heritage. Such misclassification might increase the delF508 mutation frequency. In contrast, several of the studies included in the CF Consortium dataset carefully documented the racial/ethnic origin of both parents. This brings into question which of the two estimates should be used. The lower estimate may be more correct for this racial/ethnic group. However, the higher estimate may be more appropriate for use, in that it reflects the actual performance expected in a mass screening program that would rely on self-classification.

Table 3-6. Mutation Frequencies for Hispanic Caucasians Within the Recommended 25 Mutation Panel

Num	Mutation	Mutation Frequency (%)		Average (%)	Cumulative (%)
		CF Consortium [1]	CF Foundation [2]		
1	delF508	**45.51**	**63.25**	54.38	54.38
2	G542X	**5.11**	**5.09**	5.10	59.48
8	delI507	0.59	**5.02**	2.81	62.29
22	R334W	2.25	**1.31**	1.78	64.07
6	N1303K	**1.65**	**1.67**	1.66	65.73
10	3849+10kbC>T	1.60	**1.53**	1.57	67.30
7	R553X	0.63	**0.73**	0.68	67.98
5	W1282X	0.53	**0.73**	0.63	68.61
19	R1162X	0.57	0.58	0.58	69.19
3	G551D	0.31	**0.80**	0.56	69.75
12	1717-1G>T	*0.10*	0.44	0.27	70.02
4	621+1G>T	0.00	0.51	0.26	70.28
14	711+1G>T	*0.10*	0.36	0.23	70.51
18	G85E	*0.10*	0.36	0.23	70.74
11	2789+5G>A	*0.10*	0.22	0.16	70.90
13	R347P	*0.10*	0.22	0.16	71.06
20	2184delA	*0.10*	0.22	0.16	71.22
24	3120+1G>T	*0.10*	0.22	0.16	71.38
17	3569delC	*0.10*	0.15	0.13	71.51
9	R117H	0.00	0.22	0.11	71.62
23	I148T	*0.10*	0.07	0.09	71.71
25	1078delT	*0.10*	0.07	0.09	71.80
16	A455E	*0.10*	0.00	0.05	71.85
21	1898+1G>A	*0.10*	0.00	0.05	71.90
15	R560T	0.00	0.00	0.00	71.90
	Sum	**59.95**	**83.77**	**71.90**	

[1] Cystic Fibrosis Genetic Analysis Consortium (Kazazian, 1994). Based on between 178 and 958 cystic fibrosis chromosomes (Appendix C)

[2] Based on a new analysis of the Cystic Fibrosis Foundation data based on 1,374 chromosomes (FitzSimmons S, personal communication, 2001 – Appendix B)

Table 3-7 shows the cumulative percentages of detectable mutations and detectable carrier couples for 1, 5, 10, 15, 20 and 25 mutations. Mutations are added in the order shown in Table 3-6 and are, therefore, only appropriate for Hispanic Caucasians. Figure 3-3 graphically displays the same data shown in Table 3-7 and also includes a comparison with data from for non-Hispanic Caucasians (Table 3-5 and Figure 3-2).

Table 3-7. A Comparison of Mutation Panel Size and Percentage of Hispanic Caucasian Carrier Couples Detected, Assuming an Analytic Sensitivity of 100 percent

Number of Mutations in the Panel [1]	Cumulative Percentage of Detectable Mutations	Cumulative Percentage of Carrier Couples Detected (Clinical Sensitivity)
1	54.4	29.6
5	65.7	43.2
10	69.7	48.6
15	70.9	50.3
20	71.6	51.3
25	71.9	51.7

[1] The order of added mutations is from Table 3-6

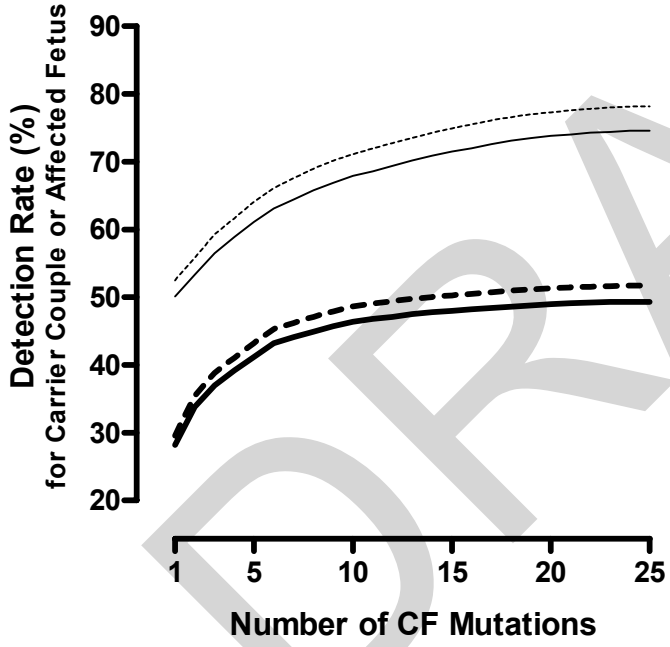

Figure 3-3. The Cumulative Percentage of Carrier Couples Detected as a Function of the Number of Mutations in the Panel. The figure is only appropriate for Hispanic Caucasians. The thick dashed line indicates the clinical specificity (detection rate), assuming an analytic sensitivity of 100 percent, while the thick solid line assumes an analytic sensitivity of 97.9 percent. The corresponding thin lines are for non-Hispanic Caucasians (from Figure 3-2).

Clinical sensitivity for cystic fibrosis among African American Couples

Description of the Mutation Frequency Data Table 3-8 contains the mutation frequencies for African Americans within the recommended panel. The two data sources are the Cystic Fibrosis Consortium and a new analysis based on the Cystic Fibrosis Foundation Patient Database. The present analysis corrects errors in the denominator of the CF Genetic Analysis Consortium data, as described for non-Hispanic Caucasians earlier in this section. The four studies selected for analysis include only individuals from the United States. None of the studies from Africa are included. A listing of the data appears in Appendix D, Table 3-17. The total numbers of chromosomes tested for each mutation range from 79 to 169. Only two of the 25 recommended mutations were not tested for, and these have been arbitrarily assigned a frequency of 0.1 percent. Cystic fibrosis is less common in African Americans and, therefore, fewer observations are available.

The CF Foundation Patient Database is summarized in Appendix B. The data are shown separately for those patients attending the TDN Centers from all self-declared African Americans in the CF Foundation Patient Database. The summary mutation frequencies are not significantly different, and the larger dataset has been chosen for analysis.

According to both datasets, the frequency of the delF508 mutation is lower in African Americans than in Caucasians. As was found in the analysis of Hispanic Caucasians, the CF Foundation Patient Database has a higher estimated frequency for this mutation (53 percent) than the CF Consortium (35 percent). This is probably due to a higher rate of admixture present among the self-declared African Americans in the CF Foundation Patient Database, compared to other studies where more extensive data about race/ethnicity were collected. Both datasets agree that the 3120+1G>T mutation is the second most common mutation among African Americans, but is relatively infrequent among Caucasians. Overall, about 65 percent of mutations among African Americans might be identifiable using the recommended panel. The lower estimate of 56 percent might be appropriate when the ethnic background is known to be exclusively African American. The higher estimate of 72 percent might be more appropriate, however, in the screening setting were self-declared race/ethnicity is relied upon. As before, the average of the results from the two data sources will be used as the best estimate.

Other studies in African Americans have been published. In a group of 82 African Americans, one study (Macek *et al*., 1997) reported that the common 'Caucasian' mutations accounted for 52 of the affected chromosomes. The addition of eight more 'African American' mutations increased the mutations identified to 75 percent. One mutation (3120+1G>A accounted for more than half of the increase and is included in the recommended panel. One other 'African American' mutations is also included (R553X) in that panel. With these two inclusions, the expected proportion of mutations detected was 66 percent, nearly identical to our estimate of 65 percent. Another study, with significant overlap with the CF Patient Database (Heim *et al*., 2001) reported that their large panels (70 and 86 mutations) would be capable of detection 81 percent of African American mutations. The recommended panel does not contain several of the mutations detected in this group and the rate of 65 percent is consistent with that reported in this study. Overall, these, and several other smaller studies report frequencies that are similar to those reported here, and their inclusion would have little impact on the overall estimates.

Table 3-8. Mutation Frequencies for African Americans Within the Recommended 25 Mutation Panel

Num	Mutation	Mutation Frequency (%) CF Consortium [1]	CF Foundation [2]	Average (%)	Cumulative (%)
1	delF508	**35.50**	**52.63**	44.07	44.07
24	3120+1G>T	**12.5**	**6.64**	9.57	53.64
8	delI507	0.74	**3.89**	2.32	55.96
7	R553X	2.37	**1.37**	1.87	57.83
2	G542X	1.18	**1.72**	1.45	59.28
3	G551D	0.59	**1.83**	1.21	60.49
4	621+1G>T	1.18	1.03	1.11	61.60
19	R1162X	0.74	0.57	0.66	62.26
22	R334W	0.74	0.23	0.49	62.75
12	1717-1G>T	0.74	0.00	0.37	63.12
6	N1303K	0.00	0.69	0.35	63.47
5	W1282X	0.00	0.47	0.24	63.71
10	3849+10kbC>T	0.00	0.34	0.17	63.88
15	R560T	0.00	0.34	0.17	64.05
18	G85E	0.00	0.23	0.12	64.17
9	R117H	0.00	0.11	0.06	64.23
13	R347P	0.00	0.11	0.06	64.29
17	3569delC	0.00	0.11	0.06	64.35
21	1898+1G>A	0.00	0.11	0.06	64.41
20	2184delA	*0.10*	0.00	0.05	64.46
23	I148T	*0.10*	0.00	0.05	64.51
11	2789+5G>A	0.00	0.00	0.00	64.51
14	711+1G>T	0.00	0.00	0.00	64.51
16	A455E	0.00	0.00	0.00	64.51
25	1078delT	0.00	0.00	0.00	64.51
	Sum	**56.46**	**72.42**	**64.51**	

[1] Cystic Fibrosis Genetic Analysis Consortium (Kazazian, 1994). Based on between 79 and 169 cystic fibrosis chromosomes (Appendix D)

[2] Based on a new analysis of the Cystic Fibrosis Foundation data -- 874 cystic fibrosis chromosomes (FitzSimmons s, personal communication, 2001 – Appendix B)

Table 3-9 shows the cumulative percentage of detectable mutations and the carrier couple detection rate for 1, 5, 10, 15, 20 and 25 mutations. Mutations are added in the order shown in Table 3-8 and are, therefore, only appropriate for African Americans. Figure 3-4 graphically displays the data shown in Table 3-9.

Table 3-9. A Comparison of Mutation Panel Size and Percentage of African American Carrier Couples Detected, Assuming an Analytic Sensitivity of 100 Percent

Number of Mutations in the Panel [1]	Cumulative Percentage of Detectable Mutations	Cumulative Percentage of Carrier Couples Detected (Clinical Sensitivity)
1	44.1	19.4
5	59.3	35.2
10	63.5	40.3
15	64.2	41.2
20	64.5	41.6
25	64.5	41.6

[1] The order of added mutations is from Table 3-8

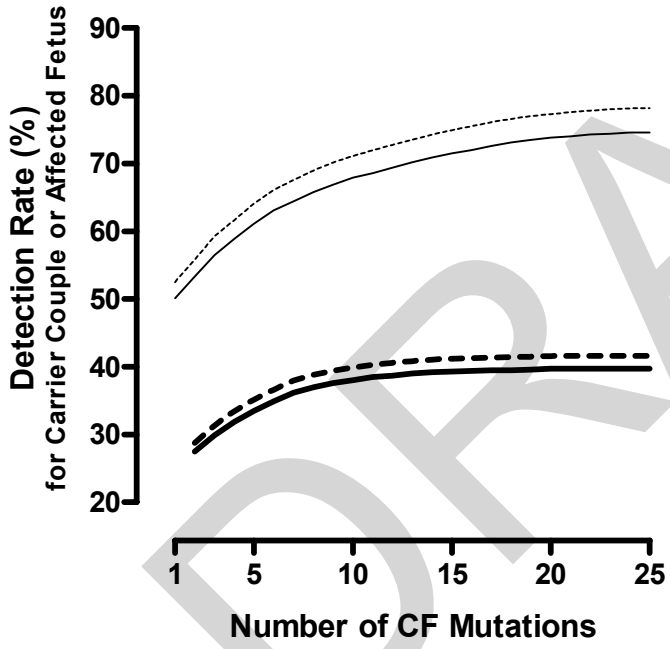

Figure 3-4. The Cumulative Percentage of Carrier Couples Detected as a Function of the Number of Mutations in the Panel. The figure is only appropriate for African Americans. The thick dashed line indicates the clinical sensitivity (detection rate), assuming an analytic sensitivity of 100 percent, while the thick solid line assumes an analytic sensitivity of 97.9 percent. The corresponding thin lines are for non-Hispanic Caucasians (from Figure 3-2).

Clinical sensitivity for cystic fibrosis among Ashkenazi Jewish couples

Several studies have reported mutation frequencies in Ashkenazi Jewish individuals with cystic fibrosis. Table 3-5 shows an analysis based on five studies reported by the Cystic Fibrosis Consortium (Kazazian, 1994). Four of these were from the United States, and one, the largest, was from Israel. Only eight mutations were identified; the most common being W1282X. Overall, about 94 percent of mutations could be identified. The data and computations associated with this analysis can be found in Appendix E. The CF Foundation Patient Database does not contain information about Ashkenazi Jewish heritage and, therefore, cannot be included in the analysis.

An earlier paper (Abeliovich *et al.,* 1992) reported that 97 percent of mutations were identified in 45 Ashkenazi Jewish individuals with cystic fibrosis using a panel of 11 mutations. This is somewhat higher than the 94.1 percent found in the present analysis which is based on the larger CF Consortium dataset. The data from this smaller study are likely to have been included in the CF Consortium report as part of the 500 chromosomes reported from Israel. The higher rate of 97 percent has been widely quoted (NIH, Grody *et al.,* 2001). A more recent report (Hiem *et al.,* 2001) reported that 95.4 percent of mutations were identified in 24 Ashkenazi Jewish individuals with cystic fibrosis using a panel of between 70 and 86 mutations. This is consistent with the present estimate of 94.1 percent. The difference between 94 and 97 percent of mutations identified is small, but the important comparison is the percentage of carrier couples detected using these two estimates. The lower estimate yields a couples detection rate of 88 percent, while the higher estimate yields a rate of 94 percent.

Several studies have documented that this distribution of mutations is only appropriate for Ashkenazi Jews (Kerem *et al.,* 1995; Kerem *et al.,* 1997; Orgad *et al.,* 2001). Non-Ashkenazi Jewish individuals usually have a lower proportion of mutations detected.

Table 3-10. Mutation Frequencies for Ashkenazi Jewish from the Cystic Fibrosis Consortium

Num	Mutation	Mutation Frequency (%)	Cumulative (%)
5	W1282X	**45.92**	45.92
1	delF508	**31.41**	77.33
2	G542X	**7.55**	84.88
10	3849+10kbC>T	**4.77**	89.65
6	N1303K	**2.78**	92.43
12	1717-1G>T	0.67	93.10
7	R553X	0.22	93.32
3	G551D	0.22	93.54
24	3120+1G>T	*0.10*	93.64
21	1898+1G>A	*0.10*	93.74
20	2184delA	*0.10*	93.84
23	I148T	*0.10*	93.94
11	2789+5G>A	*0.10*	94.04
14	711+1G>T	*0.10*	94.14
8	delI507	0.00	94.14
19	R1162X	0.00	94.14
22	R334W	0.00	94.14
4	621+1G>T	0.00	94.14
15	R560T	0.00	94.14
18	G85E	0.00	94.14
9	R117H	0.00	94.14
13	R347P	0.00	94.14
17	3569delC	0.00	94.14
16	A455E	0.00	94.14
25	1078delT	0.00	94.14
	Sum	**94.14**	

[1] Cystic Fibrosis Genetic Analysis Consortium (Kazazian, 1994). Based on between 57 and 503 cystic fibrosis chromosomes (Appendix E)

Table 3-11 shows the cumulative percentage of detectable mutations and the carrier couple detection rate for 1, 5, 10, 15, 20 and 25 mutations. Mutations are added in the order shown in Table 3-10 and are, therefore, only appropriate for Ashkenazi Jewish couples. Figure 3-5 graphically displays the data shown in Table 3-11.

Table 3-11. A Comparison of Mutation Panel Size and Percentage of Ashkenazi Jewish Carrier Couples Detected, Assuming an Analytic Sensitivity of 100 percent

Number of Mutations In the Panel [1]	Cumulative Percentage of Detectable Mutations	Cumulative Percentage of Carrier Couples Detected (Clinical Sensitivity)
1	45.9	21.1
5	92.4	85.4
10	93.7	87.8
15	94.1	88.5
20	94.1	88.5
25	94.1	88.5

[1] The order of added mutations is from Table 3-10

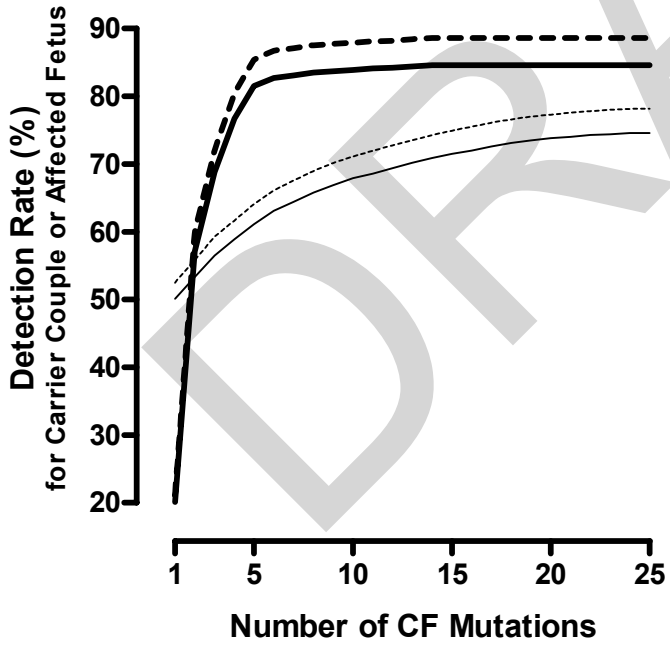

Figure 3-5. The Cumulative Percentage of Carrier Couples Detected as a Function of the Number of Mutations in the Panel. The figure is only appropriate for Ashkenazi Jewish individuals. The thick dashed line indicates the clinical sensitivity (detection rate), assuming an analytic sensitivity of 100 percent, while the thick solid line assumes an analytic sensitivity of 97.9 percent. The corresponding thin lines are for non-Hispanic Caucasians (from Figure 3-2).

Clinical sensitivity for cystic fibrosis among Asian American couples

Cystic Fibrosis is rare in Asians and, therefore, there is little information about the mutation frequencies. The Cystic Fibrosis Foundation Patient Database contains test results for 66 Asian chromosomes. It is likely that many of these individuals are not entirely of Asian background. In an earlier report from the CF Foundation, (Hamosh *et al.,* 1998), a follow-up inquiry to obtain details of one center's data revealed that '4 of the 5 Asians with cystic fibrosis had one Caucasian parent'. In another study of three Asians with cystic fibrosis, one had an American Caucasian father (Macek *et al.,* 1997) and one of the remaining cases was a product of a consanguineous relationship. In a relatively large study of 10 Asian individuals with cystic fibrosis (Heim *et al.,* 2001) only about one-quarter of the mutations were identified by the panel of 25. It is not clear whether careful examination of ethnic heritage was undertaken or whether race was 'self-declared'. Although a confident estimate for the proportion of mutations detected among Asian Americans is not possible, two findings are clear. It is likely that a large proportion of self-declared Asian individuals with cystic fibrosis in the United States will be a product of Asian and Caucasian parents. The overall proportion of mutations detected is likely to be lower for this racial/ethnic group than for any of the others studies so far.

Table 3-12. Mutation Frequencies for Asian Americans from Two Studies

Num	Mutation	Mutation Frequency (%)		Average (%)	Cumulative (%)
		Heim et al. 01 [1]	CF Foundation [2]		
1	delF508	18.80	59.09	38.95	38.95
10	3849+10kbC>T	0.00	10.61	5.31	44.26
3	G551D	6.30	0.00	3.15	47.41
6	N1303K	0.00	1.52	0.76	48.17
8	delI507	0.00	1.52	0.76	48.93
2	G542X	0.00	0.00	0.00	48.93
4	621+1G>T	0.00	0.00	0.00	48.93
5	W1282X	0.00	0.00	0.00	48.93
7	R553X	0.00	0.00	0.00	48.93
9	R117H	0.00	0.00	0.00	48.93
11	2789+5G>A	0.00	0.00	0.00	48.93
12	1717-1G>T	0.00	0.00	0.00	48.93
13	R347P	0.00	0.00	0.00	48.93
14	711+1G>T	0.00	0.00	0.00	48.93
15	R560T	0.00	0.00	0.00	48.93
16	A455E	0.00	0.00	0.00	48.93
17	3569delC	0.00	0.00	0.00	48.93
18	G85E	0.00	0.00	0.00	48.93
19	R1162X	0.00	0.00	0.00	48.93
20	2184delA	0.00	0.00	0.00	48.93
21	1898+1G>A	0.00	0.00	0.00	48.93
22	R334W	0.00	0.00	0.00	48.93
23	I148T	0.00	0.00	0.00	48.93
24	3120+1G>T	0.00	0.00	0.00	48.93
25	1078delT	0.00	0.00	0.00	48.93
	Sum	**25.10**	**72.74**	**48.93**	

[1] Analysis based on 20 chromosomes

[2] Based on a new analysis of the Cystic Fibrosis Foundation data -- 66 cystic fibrosis chromosomes (FitzSimmons S, personal communication, 2001)

Table 3-13 shows the cumulative percentage of detectable mutations and the carrier couple detection rate for 1, 5, 10, 15, 20 and 25 mutations. Mutations are added in the order shown in Table 3-12 and are, therefore, only appropriate for Asian Americans. Figure 3-6 graphically displays the data shown in Table 3-13.

Table 3-13. A Comparison of Mutation Panel Size and Percentage of Asian American Carrier Couples Detected, Assuming an Analytic Sensitivity of 100 percent

Number of Mutations In the Panel [1]	Cumulative Percentage of Detectable Mutations	Cumulative Percentage of Carrier Couples Detected (Clinical Sensitivity)
1	38.9	15.1
5	48.9	23.9
10	48.9	23.9
15	48.9	23.9
20	48.9	23.9
25	48.9	23.9

[1] The order of added mutations is from Table 3-12

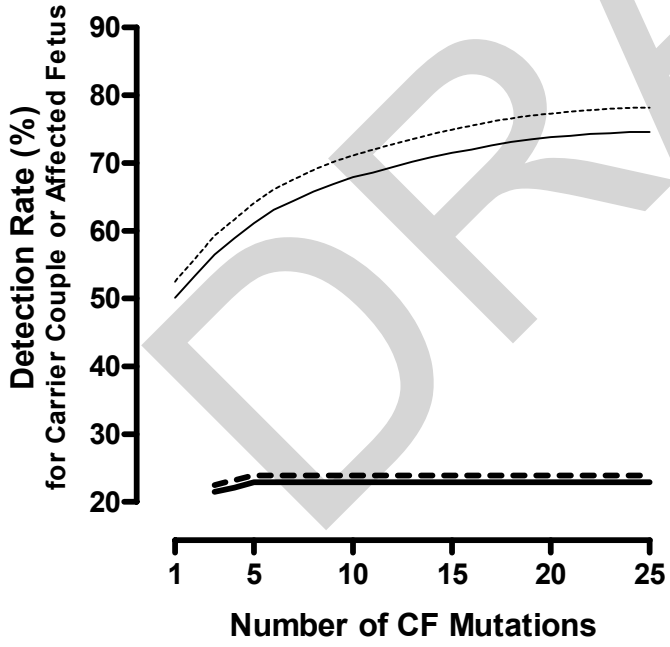

Figure 3-6. The Cumulative Percentage of Carrier Couples Detected as a Function of the Number of Mutations in the Panel. The figure is only appropriate for Asian Americans. The thick dashed line indicates the clinical sensitivity (detection rate), assuming an analytic sensitivity of 100 percent, while the thick solid line assumes an analytic sensitivity of 97.9 percent. The corresponding thin lines are for non-Hispanic Caucasians (from Figure 3-2).

References

Abeliovich D, Lavon IP, Lerer I, Cohen T, Springer C, Avital A, Cutting GR. 1992. Screening for five mutations detects 97% of cystic fibrosis (CF) chromosomes and predicts a carrier frequency of 1:29 in the Jewish Ashkenazi population. *Am J Hum Genet* **51**:951-956.

Grody WW, Cutting GR, Klinger KW, Richards CS, Watson MS, Desnick RJ. 2001. Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. *Genet Med* **3**:149-154.

Hamosh A, FitzSimmons SC, Macek M, Knowles MR, Rosenstein BJ, Cutting GR. 1998. Comparison of the clinical manifestations of cystic fibrosis in black and white patients. *J Pediatr* **132**:255-259.

Heim RA, Sugarman EA, Allitto BA. 2001. Improved detection of cystic fibrosis mutations in the heterogeneous US population using an expanded, pan-ethnic mutation panel. *Genet Med* **3**:168-176.

Kerem B, Chiba-Falek O, Kerem E. 1997. Cystic fibrosis in Jews: Frequency and mutation distribution. *Genet Test* **1**:35-39.

Kerem E, Kalman YM, Yahav Y et al. 1995. Highly variable incidence of cystic fibrosis and different mutation distribution among different Jewish ethnic groups in Israel. *Hum Genet* **96**:193-197.

Kazazian HH for the Cystic Fibrosis Genetic Analysis consortium. 1994. Population variation of common cystic fibrosis mutations. *Hum Mutat* **4**:167-177.

Macek M, Mackova A, Hamosh A et al. 1997. Identification of common cystic fibrosis mutations in African-Americans with cystic fibrosis increases the detection rate to 75%. *Am J Hum Genet* **60**:1122-1127.

Macek M, Mercier B, Mackova A, Miller PW, Hamosh A, Ferec C, Cutting GR. 1997. Sensitivity of the denaturing gradient gel electrophoresis technique in detection of known mutations and novel Asian mutations in the CFTR gene. *Hum Mutat* **9**:136-147.

Macek M, Hamosh A, Kiesewetter S, McIntosh I, Rosenstein BJ, Cutting GR. 1992. Identification of a novel nonsense mutation (L88X) in exon 3 of the cystic fibrosis transmembrane conductance regulator gene in a native Korean cystic fibrosis chromosome. *Hum Mutat* **1**:501-502.

Orgad S, Neumann S, Loewenthal R, Netanelov-Shapira I, Gazit E. 2001. Prevalence of cystic fibrosis mutations in Israeli Jews. *Genet Test* **5**:47-52.

Appendix A. Published Mutation Frequency Data Derived from the Cystic Fibrosis Genetic Analysis Consortium Data for non-Hispanic Caucasians in North America

Table 3-14 contains a reanalysis of the Cystic Fibrosis Genetic Analysis Consortium Data for non-Hispanic Caucasians (Kazazian, 1994). For easy reference, the study sites are listed in the same order as in that publication. Some studies have been reclassified to satisfy the aim of limiting the analysis to studies of non-Hispanic Caucasians. The mutations are also arranged as published. Three mutations are included in this table that are not part of the recommended prenatal panel, and three other mutations are present that are part of the panel. The table spans three pages.

Table 3-14. Reanalysis of Mutation Frequencies from the CF Genetic Analysis Consortium

Study Site	Number of Chrom	Cystic Fibrosis Mutation							
		G85E	R117H	621+1G	711+1G	1078	R334W	R347P	A455E
CAN, Toro	1034	7	13	18	5	1	4	1	4
CAN, Que	444	2		57	13				18
CAN, Que	66	0	0	9	2		0	0	0
USA, IG	1117	2	10	12		0	0	6	0
USA, NC	1083	4	15	12			0	8	1
USA MN	789								
USA, CO	600		3	9					
USA, MD	468		6	5	0	0	1	1	1
USA, TX	441		3	6	0	0	0	1	1
USA, NB	374		1	2			1	3	
USA, MI	344		1					5	
USA, MA	319		2	2					
USA, NY	319		1	2			1		
USA, IL	310			2					
USA, MA	270		2	4					
USA, PA	225	1	0	7				0	
USA, MI	216								
USA, MN	206			0					
USA, WA	194								
USA, PA	189	0	2	2	0	0	0	0	0
USA, MA	177			3					
USA, MD	142		1					1	
USA, NY	133		0	0			0		
USA, TX	130								
USA, NY	82		1	0				0	0
USA, LA	56	0	0	0	1	0	0	0	0
USA, CA	34								
USA, MA	30	0	0	0	0	0	0	0	2
Total	9792	16	61	152	21	1	7	26	27
Uncorrected Rate		0.16%	0.62%	1.55%	0.21%	0.01%	0.07%	0.27%	0.28%
Chromosome Denominator		4244	7292	7943	2728	3335	5310	5651	5010
Corrected Rate		**0.38%**	**0.84%**	**1.91%**	**0.77%**	**0.03%**	**0.13%**	**0.46%**	**0.54%**

Table 3-14. Reanalysis of Mutation Frequencies from the Cystic Fibrosis Genetic Analysis Consortium Data (Kazazian, 1994) - Continued

Study Site	Number Of Chrom	Cystic Fibrosis Mutations							
		dell507	delF508	1717-1G	G542X	S549N	G551D	R553X	R560T
CAN, Toro	1034	7	725	5	25	0	30	4	8
CAN, Que	444	1	300		2		1		
CAN, Que	66	0	47	0	0	0	0	0	0
USA, IG	1117	2	832	6	23	1	26	13	2
USA, NC	1083	3	670	6	10	0	18	11	1
USA MN	789		583	4	17		10	11	1
USA ,CO	600	0	482	2	20		11	10	1
USA, MD	468	0	314	1	11	0	10	3	1
USA, TX	441	0	328	3	12		13	6	2
USA, NB	374		253		6		6	3	1
USA, MI	344		178			1	5	7	
USA, MA	319	0	227	1	7	0	4	3	1
USA, NY	319	0	226	1	2	1	12	5	0
USA, IL	310		199		16	0	8	1	
USA, MA	270	1	181	2	12	0	10	1	0
USA, PA	225	1	155	2	11	0	4	2	2
USA, MI	216		118		2		3	1	
USA, MN	206	1	141	0	3	0	5	3	1
USA, WA	194	1	130	0	1		5	1	
USA, PA	189	0	130	1	2	1	5	0	2
USA, MA	177	1	121	3	7		4	1	1
USA, MD	142	1			1		1	1	
USA, NY	133	0	63	2	6	0	2	1	0
USA, TX	130		99		6		4	1	
USA, NY	82	0	61	3	2	0	2	1	0
USA, LA	56	0	39	0	0	0	1	0	0
USA, CA	34		28				2		
USA, MA	30	0	23	0	0	0	0	0	0
Total	9792	19	6653	42	204	4	202	90	24
Uncorrected Rate		0.19%	67.94%	0.43%	2.08%	0.04%	2.06%	0.92%	0.25%
Chromosome Denominator		7595	9650	7798	9414	6251	9792	9314	7978
Corrected Rate		**0.25%**	**68.94%**	**0.54%**	**2.17%**	**0.06%**	**2.06%**	**0.97%**	**0.30%**

Table 3-14. Reanalysis of Mutation Frequencies from the Cystic Fibrosis Genetic Analysis Consortium Data (Kazazian, 1994) - Continued

Study Site	Number of Chrom	Cystic Fibrosis Mutations							
		1889+1	2184	2789+5	R1162X	3659	3849+10	W1282X	N1303K
CAN, Toro	1034	0	3	0	2	5	4	10	8
CAN, Que	444							0	5
CAN, Que	66	0			0			0	1
USA, IG	1117		3	8	1	2	8	23	14
USA, NC	1083			9	0	5	20	20	15
USA, MN	789							4	12
USA, CO	600						2	5	5
USA, MD	468	0			0	1	1	13	5
USA, TX	441				1	1	0	3	6
USA, NB	374							1	4
USA, MI	344	2							
USA, MA	319							11	3
USA, NY	319				0		1	4	3
USA, IL	310				1		1	1	6
USA, MA	270						3	4	5
USA, PA	225				0			6	2
USA, MI	216							0	4
USA, MN	206							3	6
USA, WA	194								0
USA, PA	189	0	0	0	0	0	2	6	6
USA, MA	177							11	2
USA, MD	142								
USA, NY	133				0		2	3	5
USA, TX	130							1	2
USA, NY	82							0	2
USA, LA	56	0	2	0	0	0	0	0	0
USA, CA	34								
USA, MA	30	0	0	0	0	0	0	0	1
Total	9792	2	8	17	5	14	44	129	122
Uncorrected Rate		0.02%	0.08%	0.17%	0.05%	0.14%	0.45%	1.32%	1.25%
Chromosome Denominator		2187	2426	3509	5471	4418	6050	9078	9272
Corrected Rate		**0.09%**	**0.33%**	**0.48%**	**0.09%**	**0.32%**	**0.73%**	**1.42%**	**1.32%**

Appendix B. Mutation Frequencies for 25 CFTR Mutations According to a New Data Analysis from the Cystic Fibrosis Foundation Patient Database

Table 3-15 contains a listing of the summary data derived from the Cystic Fibrosis Foundation database for 1999 (Fitzsimmons S, personal communication 2001; Palomaki *et al.*, 2002). The study design was described earlier. This table contains the 'raw' data and the 'best' estimate for mutation frequencies may be a derivation of these numbers.

Table 3-15. Mutation Frequencies in the United States for Three Racial/Ethnic in Therapeutic Development Network (TDN) Centers and All Centers (Cystic Fibrosis Foundation)

Mutation	Number (%) of Mutations Identified					
	Non-Hispanic Caucasian		Hispanic Caucasian		African American	
	TDN Only	All	TDN Only	All	TDN Only	All
delF508	2,989 (75.90)	18,441 (75.04)	78 (60.00)	869 (63.25)	57 (47.50)	460 (59.09)
G542X	94 (2.39)	590 (2.40)	11 (8.46)	70 (5.09)	5 (4.17)	15 (1.72)
G551D	96 (2.44)	608 (2.47)	1 (0.77)	11 (0.80)	0 (0.00)	16 (1.83)
621+1G>T	48 (1.22)	227 (0.92)	1 (0.77)	7 (0.51)	1 (0.83)	9 (1.03)
W1282X	62 (1.57)	364 (1.48)	0 (0.00)	10 (0.73)	0 (0.00)	4 (0.46)
N1303K	48 (1.22)	324 (1.32)	0 (0.00)	23 (1.67)	1 (0.83)	6 (0.69)
delI507	59 (1.50)	384 (1.56)	3 (2.31)	69 (5.02)	4 (3.33)	34 (3.89)
R553X	30 (0.76)	244 (0.99)	2 (1.54)	10 (0.73)	3 (2.50)	12 (1.37)
R117H	22 (0.56)	157 (0.64)	0 (0.00)	3 (0.22)	0 (0.00)	1 (0.11)
3849+10kbC>T	17 (0.43)	158 (0.65)	3 (2.31)	21 (1.53)	1 (0.83)	3 (0.34)
2789+5G>A	19 (0.48)	86 (0.35)	0 (0.00)	3 (0.22)	0 (0.00)	0 (0.00)
1717-1G>T	16 (0.41)	123 (0.50)	0 (0.00)	6 (0.44)	0 (0.00)	0 (0.00)
R347P	17 (0.43)	66 (0.27)	0 (0.00)	3 (0.22)	0 (0.00)	1 (0.11)
711+1G>T	3 (0.08)	26 (0.11)	0 (0.00)	5 (0.36)	0 (0.00)	0 (0.00)
R560T	18 (0.46)	72 (0.29)	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.34)
A455E	5 (0.13)	43 (0.17)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
3569delC	13 (0.36)	71 (0.29)	1 (0.77)	2 (0.15)	0 (0.00)	1 (0.11)
G85E	8 (0.20)	57 (0.23)	0 (0.00)	5 (0.36)	0 (0.00)	2 (0.23)
R1162X	14 (0.36)	61 (0.25)	0 (0.00)	8 (0.58)	1 (0.83)	5 (0.57)
2184delA	9 (0.23)	36 (0.15)	0 (0.00)	3 (0.22)	0 (0.00)	0 (0.00)
1898+1G>A	9 (0.23)	47 (0.19)	0 (0.00)	0 (0.00)	1 (0.83)	1 (0.11)
R334W	6 (0.15)	29 (0.12)	2 (1.54)	18 (1.31)	0 (0.00)	2 (0.23)
I148T	3 (0.08)	15 (0.06)	1 (0.77)	1 (0.07)	0 (0.00)	0 (0.00)
3120+1G>T	2 (0.05)	6 (0.02)	0 (0.00)	3 (0.22)	8 (6.67)	58 (6.64)
1078delT	0 (0.00)	7 (0.03)	0 (0.00)	1 (0.07)	0 (0.00)	0 (0.00)
Sub Total	**3,608 (91.62)**	**22,243 (90.50)**	**103 (79.24)**	**1,151 (83.77)**	**82 (68.33)**	**633 (72.43)**
Other Identified	62 (1.57)	284 (1.16)	7 (5.38)	42 (3.06)	14 (11.67)	60 (6.86)
Not Identified	268 (6.81)	2,049 (8.34)	20 (15.38)	181 (13.17)	24 (20.00)	181 (20.71)
Total	**3,938 (100)**	**24,576 (100)**	**130 (100)**	**1,374 (100)**	**120 (100)**	**874 (100)**

Appendix C. Published Mutation Frequency Data Derived from the Cystic Fibrosis Genetic Analysis Consortium Data for Hispanic Caucasians

Table 3-16 contains a re-analysis of the Cystic Fibrosis Genetic Analysis Consortium Data for Hispanic Caucasians (Kazazian, 1994). The study sites are listed in the same order as in the original publication. The cystic fibrosis mutations are also arranged as published. Three mutations are included in this table that are not part of the recommended prenatal panel, and three other mutations are not available in that publication that are part of the panel. The table spans two pages.

Table 3-16. Reanalysis of Mutation Frequencies from the Cystic Fibrosis Genetic Analysis Consortium Data

Study Site	Number of Chrom	Cystic Fibrosis Mutation							
		G85E	**R117H**	**621+1G**	**711+1G**	**1078**	**R334W**	**R347P**	**A455E**
USA,CO	129		0	0			2		
USA,NY	49		0	0			2		
USA,TX	12								
USA,IL	10			0					
Mexico	160								
Brazil	500								
Argentina	98								
Total	958	0	0	0	0	0	4	0	0
Uncorrected Rate		0.00%	0.00%	0.00%	0.00%	0.00%	0.42%	0.00%	0.00%
Chromosome Denominator		0	178	188	0	0	178	0	0
Corrected Rate			**0.00%**	**0.00%**			**2.25%**		**0.00%**

Table 3-16 (cont.)

Study Site	Number of Chrom	Cystic Fibrosis Mutation							
		delI507	**delF508**	**1717-1G**	**G542X**	**S549N**	**G551D**	**R553X**	**R560T**
USA,CO	129	0	59	0	7		0	1	
USA,NY	49	0	22	0	2	0	2	0	0
USA,TX	12		10		0		0	0	
USA,IL	10		3		2	0	0	0	
Mexico	160	2	48		10	1	0	1	
Brazil	500		235		28		1	4	0
Argentina	98		59		0		0	0	
Total	958	2	436	0	49	1	3	6	0
Uncorrected Rate		0.21%	45.51%	0.00%	5.11%	0.10%	0.31%	0.63%	0.00%
Chromosome Denominator		338	958	178	958	219	958	958	549
Corrected Rate		**0.59%**	**45.51%**	**0.00%**	**5.11%**	**0.46%**	**0.31%**	**0.63%**	**0.00%**

Table 3-16 (cont.)

Study Site	Number of Chrom	Cystic Fibrosis Mutation							
		1889+1	**2184**	**2789+5**	**R1162X**	**3659**	**3849+10**	**W1282**	**N1303K**
USA,CO	129				2		3	1	0
USA,NY	49				0		0	0	3
USA,TX	12								
USA,IL	10				0		0	0	0
Mexico	160				0				0
Brazil	500								11
Argentina	98								
Total	958	0	0	0	2	0	3	1	14
Uncorrected Rate		0.00%	0.00%	0.00%	0.21%	0.00%	0.31%	0.10%	1.46%
Chromosome Denominator		0	0	0	348	0	188	188	848
Corrected Rate					**0.57%**		**1.60%**	**0.53%**	**1.65%**

Appendix D. Published Mutation Frequency Data Derived from the Cystic Fibrosis Genetic Analysis Consortium Data for African Americans

Table 3-17 contains a re-analysis of the Cystic Fibrosis Genetic Analysis Consortium Data for African Americans (Kazazian, 1994). The study sites are listed in the same order as in the original publication. The cystic fibrosis mutations are also arranged as published. Three mutations are included in this table that are not part of the recommended prenatal panel, and three other mutations are not available in that publication that are part of the panel. The table spans two pages.

Table 3-17. Reanalysis of Mutation Frequencies from the Cystic Fibrosis Genetic Analysis Consortium Data

Study Site	Number of Chrom	Cystic Fibrosis Mutation							
		G85E	R117H	621+1G	711+1G	1078	R334W	R347P	A455E
USA, MD	79	0	0	0	0	0	0	0	0
USA, NC	33		0	0			1	0	
USA, IL	32			1					
USA, NY	25		0	1			0		
Total	169	0	0	2	0	0	1	0	0
Uncorrected Rate		0.00%	0.00%	1.18%	0.00%	0.00%	0.59%	0.00%	0.00%
Chromosome Denominator		79	137	169	79	79	137	112	79
Corrected Rate		**0.00%**	**0.00%**	**1.18%**	**0.00%**	**0.00%**	**0.73%**	**0.00%**	**0.00%**

Table 3-17 (cont.)

Study Site	Number of Chrom	Cystic Fibrosis Mutation							
		delI507	delF508	1717-1G	G542X	S549N	G551D	R553X	R560T
USA, MD	79	0	30	0	1	1	0	3	0
USA, NC	33	1	14	1	0	0	0	0	0
USA, IL	32		8		1	0	1	1	
USA, NY	25	0	8	0	0	0	0	0	0
Total	169	1	60	1	2	1	1	4	0
Uncorrected Rate		0.59%	35.50%	0.59%	1.18%	0.59%	0.59%	2.37%	0.00%
Chromosome Denominator		137	169	137	169	169	169	169	137
Corrected Rate		**0.73%**	**35.50%**	**0.73%**	**1.18%**	**0.59%**	**0.59%**	**2.37%**	**0.00%**

Table 3-17 (cont.)

Study Site	Number of Chrom	Cystic Fibrosis Mutation							
		1889+1	**2184**	**2789+5**	**R1162X**	**3659**	**3849+10**	**W1282**	**N1303K**
USA, MD	129	0	0	0	0	0	0	0	0
USA, NC	49			0		0	0	0	0
USA, IL	12				1		0	0	0
USA, NY	10				0		0	0	0
Total	169	0	0	0	1	0	0	0	0
Uncorrected Rate		0.00%	0.00%	0.00%	0.59%	0.00%	0.00%	0.00%	0.00%
Chromosome Denominator		79	79	112	136	112	169	169	169
Corrected Rate		**0.00%**	**0.00%**	**0.00%**	**0.74%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**

The mutation frequency for 3120+1G>T was reported in Table 3-3 as being found on 14 of 112 chromosome examined (Studies from NC and MD).

Appendix E. Published Mutation Frequency Data Derived from the Cystic Fibrosis Genetic Analysis Consortium Data for Ashkenazi Jewish

Table 3-18 contains a re-analysis of the Cystic Fibrosis Genetic Analysis Consortium Data for Ashkenazi Jewish (Kazazian, 1994). The study sites are listed in the same order as in the original publication. The cystic fibrosis mutations are also arranged as published. Three mutations are included in this table that are not part of the recommended prenatal panel, and three other mutations are not available in that publication that are part of the panel. The table spans two pages.

Table 3-18. Reanalysis of Mutation Frequencies from the Cystic Fibrosis Genetic Analysis Consortium Data

Study Site	Number of Chrom	Cystic Fibrosis Mutation							
		G85E	R117H	621+1G	711+1G	1078	R334W	R347P	A455E
USA,NY	156		0	0			0		
USA,TX	57		0	0		0	0	0	0
USA,NY	38								
USA,IL	14								
Israel (Ash)	238	0	0						
Total	503	0	0	0	0	0	0	0	0
Uncorrected Rate		0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Chromosome Denominator		238	451	213	0	57	213	57	57
Corrected Rate		**0.00%**	**0.00%**	**0.00%**	**0%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**

Table 3-18 (cont.)

Study Site	Number of Chrom	Cystic Fibrosis Mutation							
		delI507	delF508	1717-1G	G542X	S549N	G551D	R553X	R560T
USA,NY	156	0	59	1	8	0	1	1	0
USA,TX	57	0	15	0	3		0	0	0
USA,NY	38		15		5				
USA,IL	14		5		1	0	0	0	
Israel (Ash)	238	0	64	2	21	0	0	0	0
Total	503	0	158	3	38	0	1	1	0
Uncorrected Rate		0.00%	31.41%	0.60%	7.55%	0.00%	0.20%	0.20%	0.00%
Chromosome Denominator		451	503	451	503	408	465	465	451
Corrected Rate		**0.00%**	**31.41%**	**0.67%**	**7.55%**	**0%**	**0.22%**	**0.22%**	**0.00%**

Table 3-18 (cont.)

Study Site	Number of Chrom	Cystic Fibrosis Mutation							
		1889+1	**2184**	**2789+5**	**R1162X**	**3659**	**3849+10**	**W1282**	**N1303K**
USA,NY	156				0		7	62	3
USA,TX	57				0	0	0	34	1
USA,NY	38						1	16	1
USA,IL	14				0		1	3	0
Israel (Ash)	238						15	116	9
Total	503	0	0	0	0	0	24	231	14
Uncorrected Rate		0.00%	0.00%	0.00%	0.00%	0.00%	4.77%	45.92%	2.78%
Chromosome Denominator		0	0	0	227	57	503	503	503
Corrected Rate		0%	0%	0%	0.00%	0.00%	4.77%	45.92%	2.78%

CLINICAL VALIDITY

Question 20: Are there methods to resolve false positive results in a timely manner?

Summary

Clinical false positives occur when two mutations are found in the fetus, but the phenotype is not classic cystic fibrosis.

Three of the less common mutations in the recommended screening panel are often not associated with the classic phenotype

- R117H occurs about 20 times more often in the general population than expected. A well-defined protocol exists to identify clinical false positive results
- D1152H occurs about 100 times more often in the Ashkenazi Jewish population than expected. No method exists for resolving clinical false positive results.
- I148T occurs about 100 times more often in the general population than expected. No method exists for resolving clinical false positive results.

One definition of a clinical false positive result would be a fetus with two of the mutations contained in the recommended panel that would not develop the phenotype generally associated with cystic fibrosis. Several of the less common mutations in the recommended panel are not associated with classic cystic fibrosis most of time. The best known of these is R117H. In the early 1990s it was recognized that too many R117H mutations were being identified in the general population, based on the known frequency of that mutation among affected individuals (Witt *et al.*, 1992). Since then, it has been discovered that the chromosomal background is an important factor in the phenotypic expression of this mutation (Kiesewetter *et al.*, 1993). Currently, a well described protocol exists (Grody *et al.*, 2001) to identify those in whom the R117H mutation is likely to be associated with classic cystic fibrosis (when combined with another deleterious mutation). That protocol can also help to identify those likely to have offspring with other very mild or normal phenotypes (Question 24).

The I148T mutation is also found too often in the general population. This mutation is now known to exist in the compound heterozygous state in asymptomatic individuals. In one study (Rohlfs *et al.*, 2001), five adult were identified with the delF508/I148T genotype who had been referred for prenatal screening; all were asymptomatic for cystic fibrosis. That same study reported that the I148T mutation accounted for 6.4 percent of 1,754 mutations identified among 42,784 individuals without cystic fibrosis (NB: This higher than expected rate has been confirmed by D Witt in a presentation to ASHG in 2001). This is in contrast to I148T being identified in 0.06 percent of the 9,236 chromosomes from individuals with cystic fibrosis (Rohlfs *et al.*, 2001). The well described protocol that is useful in determining the phenotype associated with R117H (referred to above) was not helpful in determining phenotype for this mutation. Thus, it appears that 99 of 100 I148T mutations are not associated with disease. Currently, there is no method to resolve clinical false positives when the I148T mutations are present in the fetus.

Although the mutation D1152H is not in the recommended panel, it is included in several commercial products and will, therefore, be part of the testing panel used by some screening

laboratories in the United States. D1152H is an infrequent finding among Jewish individuals with classic cystic fibrosis. In a comprehensive study of cystic fibrosis patients in Israel (Kerem *et al.*, 1995), no D1152H mutations were identified among 261 chromosomes from Ashkenazi Jewish patients and two D1152H mutations were found among 105 chromosomes from non-Ashkenazi Jewish patients. When testing the general population of Ashkenazi Jewish individuals for carrier status (Orgad *et al.*, 2001), one study found that 18 percent of all mutations identified were D1152H. Clearly, many of these mutations are not associated with classic cystic fibrosis, and the phenotype of a compound heterozygote with D1152H is likely to be normal. It is not yet clear whether over-representation of this mutation exists outside of the Jewish population.

Gap in Knowledge: Genotype/Phenotype Relationships in I148T or D1152H Compound Heterozygotes.
These two mutations are found in carrier individuals much more often than expected and are, therefore, most often associated with a normal phenotype. Currently, however, it is not possible to predict the phenotype in compound heterozygotes.

References

Grody WW, Cutting GR, Klinger KW, Richards CS, Watson MS, Desnick RJ. 2001. Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. *Genet Med* **3**:149-154.

Karem E, Kalman YM, Yahav Y et al. 1995. Highly variable incidence of cystic fibrosis and different mutation distribution among different Jewish ethnic groups in Israel. *Hum Genet* **96**:193-197.

Kiesewetter S, Macek M, Davis C et al. 1993. A mutation in CFTR produces different phenotype depending on chromosomal background. *Nat Genet* **5**:274-278.

Orgad S, Neumann S, Loewenthal R, Netanelov-Shapira, Gazit E. 2001. Prevalence of cystic fibrosis mutations in Israeli Jews. *Genet Test* **5**:47-52.

Witt DR, Blumberg B, Schaefer C et al. 1992. Cystic fibrosis carrier screening in a prenatal population. *Am J Hum Genet* **51**:A16.

Rohlfs EM, Shou Z, Sugarman EA, Heim RA, Pace RG, Knowles MR, Silverman LM, Allitto BA. 2001. The I148T CFTR mutation confers a variable phenotype and occurs on multiple haplotypes. *Am J Hum Genet* **69**:A2600

CLINICAL VALIDITY

Question 21: What is the birth prevalence of cystic fibrosis in the prenatal setting?

Summary:

The birth prevalence of cystic fibrosis in non-Hispanic Caucasians
based on all 13 published prenatal screening trials
- is **1:2488** with little difference between the US and UK estimates
- after adjusting for mutations tested and racial/ethnic heritage

based on all 17 published newborn screening trials
- is **1:2516** with significantly higher rates in the UK and lower rates elsewhere
- after adjusting for racial/ethnic heritage, prenatal testing, and, in one study, a bias introduced during allocation

based on the 3 most recent published analyses from population registries
- is **1:2499** with consistent results from the UK, US and Canada
- after adjusting for racial/ethnic heritage, length of follow-up and known under-ascertainment
-

The birth prevalence of cystic fibrosis in the Ashkenazi Jewish population
based on four studies of carrier frequency and one population survey
- is **1:2271**

The birth prevalence of cystic fibrosis in Hispanic Caucasians in the United States
based on three studies of carrier frequency and one population survey
- is **1:13,535**

The birth prevalence of cystic fibrosis in African Americans
based on three population surveys
- is **1:15,100,** but two small studies of carrier frequency indicate a lower prevalence

The birth prevalence of cystic fibrosis in Asian Americans
based on one population survey
- is **1:31,000,** but studies of Asians in their native lands find much lower rates

Introduction

In the prenatal screening setting, the birth prevalence of a given disorder is defined as the number of cases that would be present at birth in the absence of prenatal diagnosis and selective termination. The birth prevalence of cystic fibrosis can be estimated from three separate data sources:

- prenatal screening trials,
- newborn screening studies,
- population-based studies and registries.

When analyzing prevalence, it is important to consider both geographical and racial/ethnic variability. The first analysis presented is restricted to non-Hispanic Caucasians of Northern or Southern European heritage, since the majority of published data deals with this group. Later, similar analyses will be provided for other groups that include:

- Ashkenazi Jewish Caucasians
- Hispanic Caucasians
- African Americans/Afro-Caribbean Blacks
- Asian Americans

Cystic fibrosis prevalence: Prenatal screening trials

Thirteen population-based prenatal screening trials have been published that can be used to estimate the prevalence of cystic fibrosis among non-Hispanic Caucasians of Northern European heritage. The estimate relies on the observed carrier rate among pregnant women being tested for a cystic fibrosis mutation (without a family history of cystic fibrosis), in combination with each individual trial's mutation detection rate. For example, a trial might report 33 carrier women identified among 1000 women tested (observed carrier rate of 0.033). That trial also reports that its laboratory uses a mutation panel that identifies 80 percent of all cystic fibrosis mutations. The carrier rate can then be corrected to 0.04125 (0.033/0.8), to take this into account. This, in turn, allows the prevalence of cystic fibrosis to be estimated at 0.0004254 (1/4 * 0.04125 * 0.04125) or about 1:2350.

Table 3-19 shows, for each of the 13 studies, the number of individuals (women and their partners) tested, the number of carriers detected, the observed carrier rate, the mutation detection rate, and the corrected carrier rate. The computed prevalence and 95% confidence intervals are listed in the last two columns. Overall, a cystic fibrosis mutation is detected in 1,233 of the 39,284 individuals tested.

Table 3-19. Estimated Cystic Fibrosis Prevalence Derived from Prenatal Screening Trials

Study Number	Persons Tested	Carriers Detected	Observed Rate	Mutations Detected (%)	Corrected Rate	CF Prevalence (1 in n)	95% CI
1	3,275	115	0.0351	85.0	0.0413	2344	1694-3322
2	6,761	175	0.0259	87.7	0.0295	4592	3522-6151
3	1,167	39	0.0303	84.5	0.0359	3110	1780-5783
4	562	19	0.0338	80.0	0.0423	2240	1071-5739
5	658	18	0.0274	73.2	0.0374	2864	1308-6719
6	4,413	160	0.0363	83.0	0.0437	2090	1606-2829
7	1,867	62	0.0332	91.0	0.0365	3004	1899-4959
8	4,210	108	0.0257	75.1	0.0342	3428	2488-4789
9A	1,091	32	0.0293	75.0	0.0391	2615	1451-4925
9B	2,633	97	0.0368	80.0	0.0461	1886	1337-2727
10	3,948	135	0.0342	80.0	0.0427	2189	1613-2970
11	3,286	74	0.0225	65.0	0.0346	3332	2323-4856
12	1,621	47	0.0290	75.0	0.0387	2676	1632-4444
13	3,792	152	0.0401	96.0	0.0418	2294	1700-3174
Total	**39,284**	**1233**					

The following is a brief discussion of how the numbers were obtained from each study listed in the table. For additional information about the outcomes of these screening trials, see Pilot Trials (Clinical Utility, Question 33).

1. *Edinburgh, Scotland* – Only the first report (Mennie *et al.*, 1992) is included in the table. In later reports, it is not possible to separate women with no family history from the

relatively large number of women with a known family history. The population is assumed to be entirely Scottish; several potential participants were not screened, due to low prevalence in their racial/ethnic group. The mutation detection rate of 85 percent was derived from a local population (Shrimpton *et al.*, 1991). Overall, 3,165 women (and 110 partners) were tested with 111 carrier women (and four carrier partners) detected.

2. *Copenhagen, Denmark* – All data from this report (Schwartz *et al.*, 1993) are included in the table. The Scandinavian countries are known to have a lower prevalence of cystic fibrosis, and this is borne out in the estimated prevalence of 1:4529, by far the lowest of the 13 studies. The mutation detection rate was derived from a local population and is contained in the original reference. None of the women tested had a family history of cystic fibrosis. Overall 6,599 women (and 162 partners) were tested, with 172 carrier women and three carrier partners identified.

3. *Manchester, England* – The data were derived from two reports of this screening program (Harris *et al.*, 1993; Hartley *et al.*, 1997). Couples with a family history were allowed into the study but were not actively recruited. The mutation detection rate was derived from an unpublished study in a local population. In the first report, 127 women (and five partners) were tested, with five carrier women detected. In addition, 117 women (and all 117 partners) were tested, with a total of eight carrier individuals detected. In the second report, 267 women (and 10 partners) were tested, with 10 carrier women detected, along with 262 women (and all 262 partners), with a total of 16 carrier individuals detected.

4. *Oxford, England* – The data were derived from two reports of this screening program (Wald *et al.*, 1993; Wald *et al.*, 1995). It is assumed that couples with a family history were allowed into the study but were not actively recruited. No reference was quoted for the mutation detection rate of 80 percent, but this estimate is similar to the 80.1 percent summary estimate published for the United Kingdom (Dequeker *et al.*, 2000). The numbers include samples tested from the male partner after a mutation was initially identified in the woman. Using the unduplicated numbers from the second report, 543 women (and 19 partners) were tested, with 19 carrier women detected.

5. *East Berlin, Germany* – The data were derived from a single report (Jung *et al.*, 1993). It is assumed that couples with a family history were allowed into the study but were not actively recruited. No reference was quoted for the mutation detection rate of 61.2 percent, which is much lower than the 73.2 percent published summary estimate for Germany (Dequeker *et al.*, 2000). The present analysis uses the 73.2 percent estimate. Overall, 637 women and 3 men were initially tested (and 18 partners), with a total of 18 women carriers and one partner carrier identified.

6. *Maine, USA* – The data were derived from two published studies (Doherty *et al.*, 1996; Bradley *et al.*, 1998) and a personal communication (Bradley, 2000). Couples with a known family history were not included. The population was 99 percent Caucasian and mainly of Northern European heritage. The mutation detection rate of 80 percent was based on data from the Cystic Fibrosis Genetic Analysis Consortium (1994). Overall,

4,260 women were tested (along with 153 partners), with a total of 153 women carriers and 7 partner carriers detected.

7. *Aberdeen, Scotland* – The data were derived from one published study (Miedzybrodzka *et al.*, 1995). The study included 12 couples with a known family history of cystic fibrosis; no adjustment is made for this high risk group. The mutation detection rate of 92 percent was derived from a local population (Miedzybrodzka *et al*, 1993). In the two-step arm of the study, 1,487 women were tested (along with 47 partners), with a total of 48 women carriers and one partner carrier detected. In the one-step arm of the study, 321 women were tested (along with 12 partners), with a total of 12 women carriers and one partner carrier detected.

8. *Rochester, New York* – The data were derived from one published study (Loader *et al*, 1996) and a personal communication (Rowley, 1998). Of the 4,879 couples successfully tested, only 4,391 were pregnant, and 109 carrier women were detected. A family history was identified in 27 participants, 4 of whom were also carriers. All 27 were removed from this analysis. A total of 96 partners were tested, and 5 carriers were detected. The population was 94.4 percent non-Hispanic Caucasian. The estimated 250 participants remaining were also removed from the analysis. It was estimated that two mutations occurred in this group. Thus, an estimated 4,210 non-Hispanic Caucasian couples without a known family history were tested, with 108 carrier individuals identified. The reported mutation detection rate of 75.1 percent was based on the Cystic Fibrosis Genetic Analysis Consortium (1994).

9. *Northern California, USA* – The data were derived from a single publication (Witt *et al.*, 1996) and a personal communication (Witt, 1998). Laboratory analysis was performed in two laboratories (A and B) with different mutation detection rates. It is for this reason that two separate results are computed for this trial. In Laboratory A, 1,091 non-Hispanic Caucasian women were tested, and 32 carrier women were identified. In Laboratory B, 2,633 non-Hispanic Caucasian women were tested, and 95 carrier women and one compound heterozygote were identified. The test results in the partners were not stratified by race and were, therefore, not included. The authors estimated mutation detection rates of 80 and 85 percent for Laboratories A and B. However, these estimates have been adjusted to 75 and 80 percent, respectively, to makes these rates consistent with those used in other pilot studies and to reflect the large proportion of non-Hispanic Caucasians in California (e.g., Italians, Greeks) who are not of northern European heritage compared to other less diverse populations (e.g., Maine).

10. *Leeds, England* –The data were derived from a single report (Cuckle *et al.*, 1996). It is assumed that couples with a family history were allowed into the study but were not actively recruited. The mutation detection rate was reported to be between 80 and 90 percent in Yorkshire, but no reference as provided. The present analysis uses 85 percent. Overall, 3,773 women (and 127 partners) were tested, and 130 carrier women and 3 carrier partners were identified. In addition, 48 men attending with their partner requested immediate testing, and two carriers were identified.

11. *Milan, Italy* – The data were derived from a single report (Brambati *et al.*, 1996). Among 2,231 parents without a family history of cystic fibrosis, 46 carrier individuals were identified. In addition, 1,055 fetuses were tested, and 26 carriers and one compound heterozygote were identified. The mutation detection rate was reported to be 65 percent, based on an unpublished report. This is consistent with the 65.2 percent published summary estimate for Italy (Dequeker *et al.*, 2000).

12. *Los Angeles, California* – The data were derived from a single report (Grody *et al.*, 1997) and a personal communication (Grody, 1998). Couples with a family history were excluded. The reported mutation detection rate is 75 percent. In this study, 47 carrier women were identified among 1,851 non-Hispanic Caucasian women tested. However, interpretation of these results is complicated by the fact that some ethnic groups were counted twice (i.e., the sum of the reported ethnic-specific prevalences reported is 270 observations higher than the total number of tests performed). In order to provide a reasonably reliable estimate, we have assumed that the Hispanic and non-Hispanic Caucasian groups accounted for 90 percent of the double-counting (Table 4 in Grody *et al.*, 1997). The double-counts are then divided between the two groups in the ratio of Hispanic to non-Hispanic study subjects. Thus, 160 ([1596 / (1596+921)) * 270 * .9] observations are subtracted from the denominator of 1,851, leaving 1,621 women tested. Among this group of non-Hispanic Caucasians, 365 (23 percent) are Ashkenazi Jewish.

13. *New York City, USA* – The data were derived from a single report (Eng *et al.*, 1997). The population was Ashkenazi Jewish Caucasians without a family history of cystic fibrosis. The mutation detection rate of 96 percent was from a published source specific to that population group. Overall, 3,792 individuals were tested, with 152 carrier individuals identified.

Except for the study from Denmark, the remaining 12 studies (13 observations) are reasonably consistent in their estimates of cystic fibrosis prevalence, even . Figure 7 shows the results of a formal meta-analysis (Berlin *et al.,* 1989). The X-axis is labeled with the study number, in increasing order from lowest to highest estimate of prevalence. The Y-axis shows the prevalence estimates (circles), along with the 95% confidence interval (vertical lines). The horizontal solid line shows the consensus estimate, with the corresponding 95% confidence interval shown by broken horizontal lines. Overall, the prevalence is estimated to be 1:2488 (95 percent CI 1:2224 to 1:2782). A formal test for heterogeneity is statistically significant ($\chi^2 = 12$, $p < 0.001$), indicating that the between-study differences in prevalence estimates are unlikely to have occurred by chance. When stratified by whether or not the studies are from the United States (5 studies, 6 observations) or from Europe (7 studies), the two estimates are still similar: 1:2403, (95 percent CI 1:1995 to 1:2894) and 1:2577 (95 percent CI 1:2192 to 1:3029), respectively. Heterogeneity is reduced, but still significant ($\chi^2 = 8$, $p = 0.005$ and $\chi^2 = 4$, $p = 0.04$).

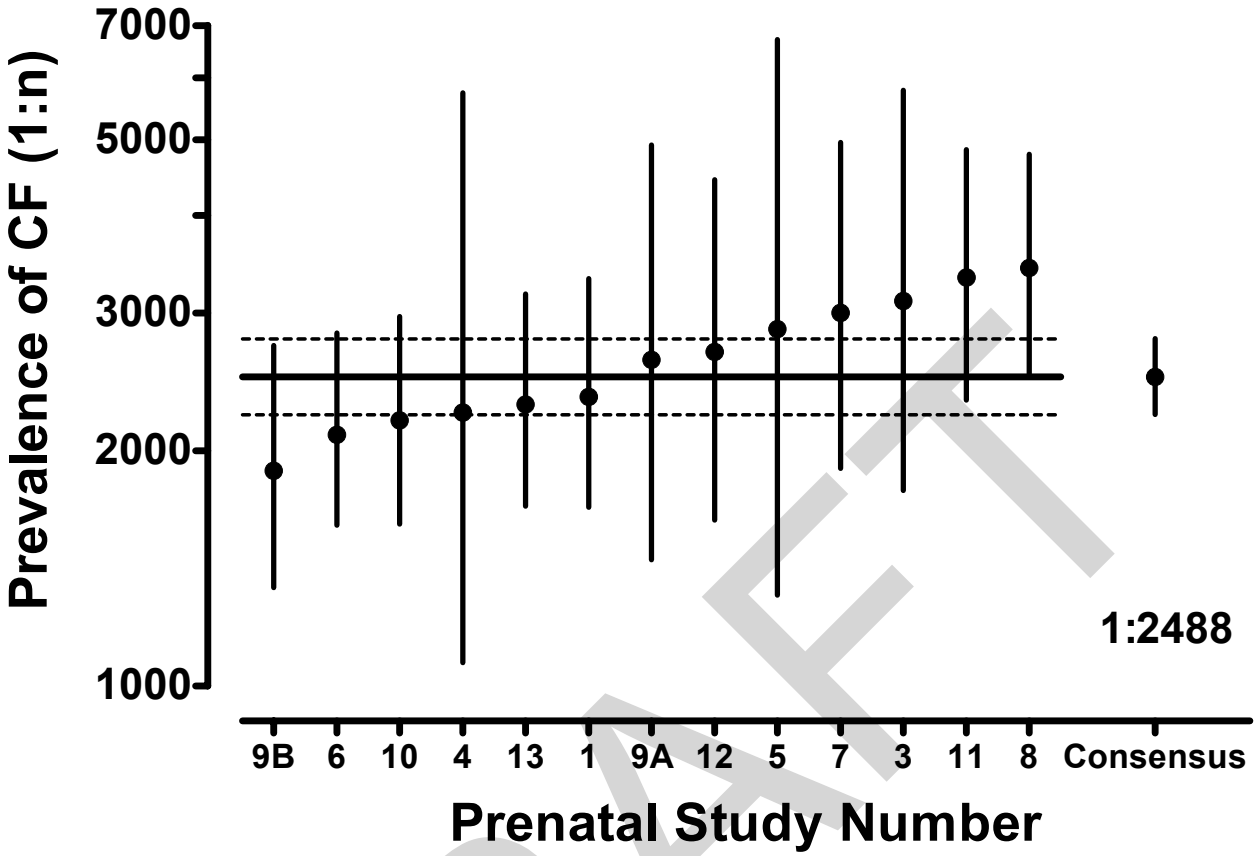

Figure 7 – Summary of Cystic Fibrosis Prevalence Estimates Derived From Prenatal Screening Trials

Strengths and weaknesses of these estimates

Family history of cystic fibrosis Most studies included only couples without a family history. These studies will tend to underestimate the prevalence of cystic fibrosis, but only by a small amount (about 2 percent). A few additional studies were corrected for the inclusion of couples with a family history, and the remainder of the studies allowed those couples to be screened. Because of this mix, the estimates provided are not likely to be significantly biased with respect to the inclusion, or exclusion, of couples with a family history of cystic fibrosis.

Using carrier rates to estimate prevalence The use of carrier rates to estimate prevalence of cystic fibrosis is reliable, if the mutations detected have high penetrance (i.e., individuals with two of the detectable mutations will have the cystic fibrosis phenotype). The majority of studies relied on relatively small mutational panels consisting of well-described mutations that are known to cause cystic fibrosis. It is important to note, however, that one study routinely tested for a less common mutation (R117H) that is known to be associated with non-cystic fibrosis phenotypes. In that study, the R117H mutation accounted for 16 percent of the carriers. In a population of individuals with cystic fibrosis, this mutation accounts for less than 1 percent of the mutations. Thus, among laboratories testing for the less common and less well described mutations, an artificially high prevalence might be anticipated. One

advantage of using this method to indirectly estimate the prevalence is that many fewer individuals need to be tested. The largest study included less than 7,000 couples. In a group that size, only two or three affected fetuses would be expected. However, when carrier rates are used to estimate prevalence, even the studies of 2,000 couples yielded individually reliable estimates.

A note on the demographic identifiers used throughout this document We realize that demographic identifiers of race and ethnicity are potentially controversial. The biological validity of *race* has been challenged by biologists, and the original definition of *ethnicity* is a category that would describe non-biological, but socially meaningful, groups. In this document, we follow the usage of the investigators whose studies we discuss. In all studies from the United States, those categories follow current Census Bureau usage. The major, relatively new element of the Census Bureau demographic categories is the addition of Hispanic as a separate category and the separation of Hispanic into "white" and "non-white" categories. While we recognize potential errors with the use of these categories, only one presents hazards for the topic at hand. That is the extrapolation of rates of cystic fibrosis disease and gene prevalence to populations which are biologically dissimilar from those in a cited study. For example, Hispanic whites in Florida could have different rates and types of mutations for cystic fibrosis from the indigenous groups in Mexico and Central America. The only remedy for this problem is to keep a high level of awareness of the potential for genetic differences between groups that would currently fall under the same rubric of demographic identification. Data on frequency and type of mutations, if collected and analyzed as part of prenatal screening for cystic fibrosis, will allow more refined estimates to be made in the future.

References

Berlin JA, Laird NM, Sacks HS, Chalmers T. 1989. A comparison of statistical methods for combining event rates from clinical trials. *Stat Med* **8**:141-151.

Bradley LA; Johnson DD; Doherty RA; Palomaki GE; Haddow JE. 1998. Routine prenatal cystic fibrosis screening in primary care offices. *Am J Hum Genet* **63**:A13.

Brambati B, Anelli MC, Tului L. 1996. Prenatal cystic fibrosis screening in a low-risk population undergoing chorionic villus sampling for fetal karyotyping. *Clin Genet* **50**:23-27.

Brock DJH. 1996. Prenatal screening for cystic fibrosis: 5 years' experience reviewed. *Lancet* **347**:148-150.

Cuckle H, Quirke P, Sehmi I, Lewis F, Murray J, Cross D, Cuckle P, Ozols B. 1996. Antenatal screening for cystic fibrosis. *Br J Obstet Gynaecol* **103**:795-799.

Doherty RA, Palomaki GE, Kloza EM, Erickson JL, Dostal DA, Haddow JE. 1994. Prenatal screening for cystic fibrosis. *Lancet* **343**:172.

Doherty RA, Palomaki GE, Kloza EM, Erickson JL, Haddow JE. 1996. Couple-based prenatal screening for cystic fibrosis in primary care settings. *Prenat Diagn* **16**:397-404.

Eng CM, Schechter C, Robinowitz J, Fulop G, Burgert T, Levy B, Zinberg R, Desnick RJ. 1997. Prenatal genetic carrier testing using triple disease screening. *JAMA* **278**:1268-1272.

Grody WW, Dunkel-Schetter C, Tatsugawa ZH, Fox MA, Fang CY, Cantor RM, *et al*. 1997. PCR-based screening for cystic fibrosis carrier mutations in an ethnically diverse pregnant population. *Am J Hum Genet* **60**:935-947.

Hartley NE, Scotcher D, Harris H, Williamson P, Wallace A, Craufurd D, *et al*. 1997. The uptake and acceptability to patients of cystic fibrosis carrier testing offered in pregnancy by the GP. *J Med Genet* **34**:459-464.

Holloway S, Brock DJH. 1994. Cascade testing for the identification of carriers of cystic fibrosis. *J Med Screen* **1**:159-164.

Jung U, Urner U, Grade K, Coutelle C. 1994. Acceptability of carrier screening for cystic fibrosis during pregnancy in a German population. *Hum Genet* **94**:19-24.

Livingstone J, Axton RA, Gilfillan A, Mennie M, Compton M, Liston WA, *et al*. 1994. Antenatal screening for cystic fibrosis: a trial of the couple model. *BMJ* **308**:1459-1462.

Loader S, Caldwell P, Kozyra A, Levenkron JC, Boehm CD, Kazazian HH, *et al*. 1996. Cystic fibrosis carrier population screening in the primary care setting. *Am J Hum Genet* **59**:234-247.

Mennie ME, Gilfillan A, Compton M, Curtis L, Liston WA, Pullen I, *et al*. 1992. Prenatal screening for cystic fibrosis. *Lancet* **340**:214-216.

Miedzybrodzka ZH, Dan JCS, Russel G, Friend JAR, Kelly KF, Haites NB. 1993. Prevalence of cystic fibrosis mutations in the Grampain region of Scotland. *J Med Genet* **30**:316-317.

Miedzybrodzka ZH, Hall MH, Mollison J, Templeton A, Russell IT, Dean JCS, *et al*. 1995. Antenatal screening for carriers of cystic fibrosis: randomised trial of stepwise *v* couple screening. *BMJ* **310**:353-357.

Rowley PT, Loader S, Levenkron JC. 1997. Cystic fibrosis carrier population screening: A review. *Genet Test* **1**:53-59.

Schwartz M, Brandt NJ, Skovby F. 1993. Screening for carriers of cystic fibrosis among pregnant women: a pilot study. *Eur J Hum Genet* **1:**239-244.

Wald NJ, George LM, Wald NM, Mackenzie IZ. 1993. Couple screening for cystic fibrosis. *Lancet* **342**:1307-1308.

Wald NJ, George L, Wald N, MacKenzi IZ. 1995. Further observations in connection with couple screening for cystic fibrosis. *Prenat Diagn* **15**:589-590.
Witt DR, Schaefer C, Hallam P, Wi S, Blumberg B, Fishbach A, *et al*. 1996. Cystic fibrosis heterozygote screening in 5,161 pregnant women. *Am J Hum Genet* **58**:823-835.

Cystic fibrosis prevalence: Newborn screening trials

Cohorts identified as part of newborn screening trials (utilizing measurements of immunoreactive trypsinogen and/or DNA testing) can also be used to estimate the prevalence of cystic fibrosis. In many of these trials, extensive efforts were made to identify false negative results, in order to quantify screening performance. The following section presents cystic fibrosis prevalence estimates for non-Hispanic Caucasians, based on results from 17 newborn screening trials. Some published trials are excluded, because they either did not attempt complete ascertainment or did not sufficiently document the diagnostic criteria or population studied. Others were not published in English or were presented in proceedings of a meeting that are difficult to obtain.

Table 3-20 contains a brief description of the 17 selected studies, carried out in four geographical areas: the United Kingdom (UK), Australia/New Zealand, the United States, and Europe (not including the UK). For each study, the inclusive dates, the location, and the ethnic make-up of the study population are provided. In addition, three possible sources of bias in ascertainment are listed. The first two summarize the compliance with follow-up screening tests (i.e., the percentage of newborns with initially high immunoreactive trypsinogen [IRT] measurements from whom a second requested sample could be obtained) and with diagnostic tests (i.e., the percentage of newborns who received a sweat test, when indicated). The last column indicates whether or not the study was likely to under-ascertain newborn cases of cystic fibrosis, due to some of the affected pregnancies being identified prenatally and selectively terminated.

Taking non-compliance into account

In a two-step IRT screening protocol, the first measure of compliance is the proportion of those with screen positive results on the first sample who submit a second sample. The second measure of compliance is the proportion of those remaining screen positive who then receive diagnostic sweat testing. In 14 of the 17 studies in Table 3-20, compliance with the protocol was high. In the other three studies, compliance at the diagnostic or follow-up screening level was between 74 and 81 percent. If the risk of cystic fibrosis were the same among newborns whose parents did not comply with the complete testing protocol as among those who were compliant, then that factor could be taken into account. If IRT levels were lower among newborns who were not fully tested, however, the risk for cystic fibrosis in that group might well be lower. Although IRT levels were not provided on these studies for the non-compliant group, all three studies attempted to identify all cases, regardless of screening status and compliance. These efforts at complete ascertainment are considered to compensate for the non-compliance. For this reason, none of these studies are corrected for compliance.

Table 3-20. Demographic and Study-Related Characteristics of Newborn Cystic Fibrosis Screening Trials

Study Number	Location	Years	Race / Ethnicity	Possible Biases		
				Compliance		Prenatal Diagnosis
				Screen	Diag	
United Kingdom						
1	Leeds	1975-94	NHC	NR	NR	Yes
2	East Anglia	1980-89	NHC	NA	NR	No
3	N. Ireland	1983-87	NHC	NR	NR	No
4	Wales/Midlands	1985-90	NHC	NR	100%	Yes
5	Trent	1989-94	NHC	99%	99%	Yes
Australia / New Zealand						
6	New South Wales	1981-93	NHC	98%	NR	Yes
7	New Zealand	1983-86	NHC	NR	NR	No
8	Victoria	1987-93	NHC	NA	100%	Yes
9	Adelaide	1989-93	NHC	NA	100%	Yes
United States						
10	Colorado	1982-87	Mixed	77%	100%	No
11	Wisconsin	1985-94	Mixed	NA	81/100%	Yes
12	W. Pennsylvania	1987-91	Mixed	NR	NR	Yes
Europe (w/o the United Kingdom)						
13	Normandy, France	1980-82	NHC	NA	NR	No
14	Vienna, Austria	1988-91	NHC	NA	74%	Yes
15	Northeastern Italy	1988-91	NHC	100%	NR	Yes
16	France	1989-90	NHC	93%	NR	Yes
17	Brittany, France	1993-99	NHC	NA	100%	Yes

NHC = non-Hispanic Caucasians; Mixed = NHC and at least one other racial/ethnic group; NR = not reported; NA = not applicable; Diag = diagnosis

Taking mixed racial/ethnic populations into account All but the three studies from the United States appear to have been conducted in populations that were nearly exclusively composed of non-Hispanic Caucasians. Since other races (along with Hispanic Caucasians) are reported to have lower prevalences of cystic fibrosis, it is important to take this factor into account. In two of the U.S. studies (Hammond *et al.*, 1991; Gregg *et al.*, 1993), cystic fibrosis prevalence was reported for both the entire population and for Caucasians alone. However, neither study analyzed prevalences separately in the Hispanic or Asian populations. These two groups are estimated to have much lower prevalences (about 1:9000 and 1:30,000, respectively). The third study reported that both Blacks and other racial/ethnic groups were included but provided no separate estimates. None of the three studies provided racial/ethnic breakdowns for the affected and unaffected populations. The present analysis

deals with this issue by deriving estimates of the racial/ethnic distribution for each study from the National Center for Health Statistics (National Center for Health Statistics 1989; 1993), using a one year time period near the middle of the study. In this analysis, the total number of non-Hispanic Caucasians is derived by multiplying the total number of study subjects by the proportion estimated to be non-Hispanic Caucasian. Then, the number of newborns with cystic fibrosis is reduced by the number of cases expected in other racial/ethnic groups. This is estimated by computing the number of newborns for the other racial/ethnic groups (Black, Hispanic Caucasian and Asian) and then multiplying that number by the appropriate prevalences.

Taking the impact of prenatal diagnosis into account Of the 17 newborn screening trials summarized in Table 3-21, 12 (71 percent) were active during the time period when prenatal diagnosis was possible (1988 or later). The effect of prenatal diagnosis on the birth prevalence of cystic fibrosis depends on the percentage of pregnancies terminated after identification of a fetus with two mutations. Of these 12 studies, three from Australia/New Zealand (Ranieri *et al.*, 1994; Balnaves *et al.*, 1995; Wilcken *et al.*, 1995) and one from France (Scotet *et al.*, 2000) estimated the impact of prenatal diagnosis on the birth prevalence. One study provided the rate by year from 1987 through 1993 (Balnaves *et al.*, 1995). Overall, these trials identified 80 prenatal diagnoses and selective terminations of fetuses with cystic fibrosis in the cohort who would otherwise have been tested as newborns. Three of these trials were active only after 1988; the fourth (Wilcken *et al.*, 1995) screened roughly half of the newborns after 1988. Dividing the total number screened in these four trials by the number of prenatal diagnoses can provide a rough approximation of the impact of prenatal diagnosis for those studies not providing such data. Based on this, approximately one prenatal diagnosis can be expected for every 16,800 newborns tested (1,258,381 / 80). Studies reporting results since 1988 that did not account for prenatal diagnosis are corrected by this factor. Under this assumption, a study that tested 168,000 newborns since 1988 would have identified 10 additional newborns with cystic fibrosis, had prenatal diagnosis not been available. If studies were active both before and after 1988, the number of newborns tested after 1988 is estimated assuming a uniform recruitment rate (unless yearly recruitment was provided). It is possible that prenatal diagnosis is less common in the United States and this correction will over estimate the prevalence.

Table 3-21 shows the total number of newborns screened for each of the studies (numbers assigned in Table 3-20), along with the total number of cases identified. The last four columns contain the corrected numbers tested and cases identified, along with an estimate of the prevalence and the corresponding 95% confidence intervals. Corrections for individual studies are described in text following the table. Below each of the four geographical groups is a summary prevalence, computed using a random effects model (Berlin *et al*., 1989). At the bottom of the table is the summary prevalence for all studies. Figure 3-8 shows the same data in graphical form. The overall estimate for prevalence in non-Hispanic Caucasians is 1:2509, but there is considerable heterogeneity between studies ($\chi^2 = 35.0$, $p < 0.001$). This is greatly reduced when the results are stratified by geographic region. Even then, however, heterogeneity exists in the prevalence estimates from the United Kingdom and Europe.

Table 3-21. Reported and Adjusted Cystic Fibrosis Prevalence in Non-Hispanic Caucasians, Derived from Newborn Screening Studies

Study Number	Reported		After Adjustment			
	Tested	Cases (%MI)[1]	Tested	Cases	Prevalence	95% CI
United Kingdom						
1	81,778	37 (14)	81,778	37	1:2210	1604-3139
2	211,344	98 (18)	211,344	98	1:2157	1792-2706
3	108,422	70 (17)	108,422	70	1:1549	1226-1987
4	227,183	78 (8)	227,183	78+14	1:2469	2041-3125
5	437,959	170 (14)	437,959	170+26	1:2234	1956-2561
Subtotal	$\chi^2 = 6.3$, p = 0.01		**1,066,696**		**1:2123**	**1838-2451**
Australia / New Zealand						
6	1,204,000	451 (20)	1,204,000	451+24	1:2535	2323-2789
7	210,751	78 (24)	210,751	78	1:2702	2165-3418
8	309,873	112 (16)	309,873	112+32	1:2152	1844-2533
9	88,752	29 (24)	88,752	29+6	1:2536	1823-3640
Subtotal	$\chi^2 = 2.7$, p = 0.1		**1,813,376**		**1:2465**	**2259-2689**
United States						
10	279,399	73 (16)	181,552	73-7	1:2751	2162-3556
11	325,173	82 (21)	285,502	82-3+17	1:2974	2467-3743
12	105,734	20 (NR)	86,702	20-1+5	1:3613	2428-5637
Subtotal	$\chi^2 = 0.8$, p = 0.5		**553,753**		**1:2963**	**2480-3540**
Europe (w/o United Kingdom)						
13	78,800	23 (13)	78,800	23	1:3426	2283-5405
14	19,882	12 (NR)	19,882	12+1	1:1538	909-2888
15	157,992	42 (NR)	157,992	42+9	1:3098	2356-4160
16	513,440	122 (12)	513,440	122+31	1:3356	2889-4003
17	343,756	118 (14)	343,756	118+18	1:2528	2157-3052
Subtotal	$\chi^2 = 7.7$, p = 0.01		**1,113,870**		**1:2875**	**2359-3503**
Total	$\chi^2 = 35.0$, p < 0.001		**4,547,695**		**1:2509**	**2286-2754**

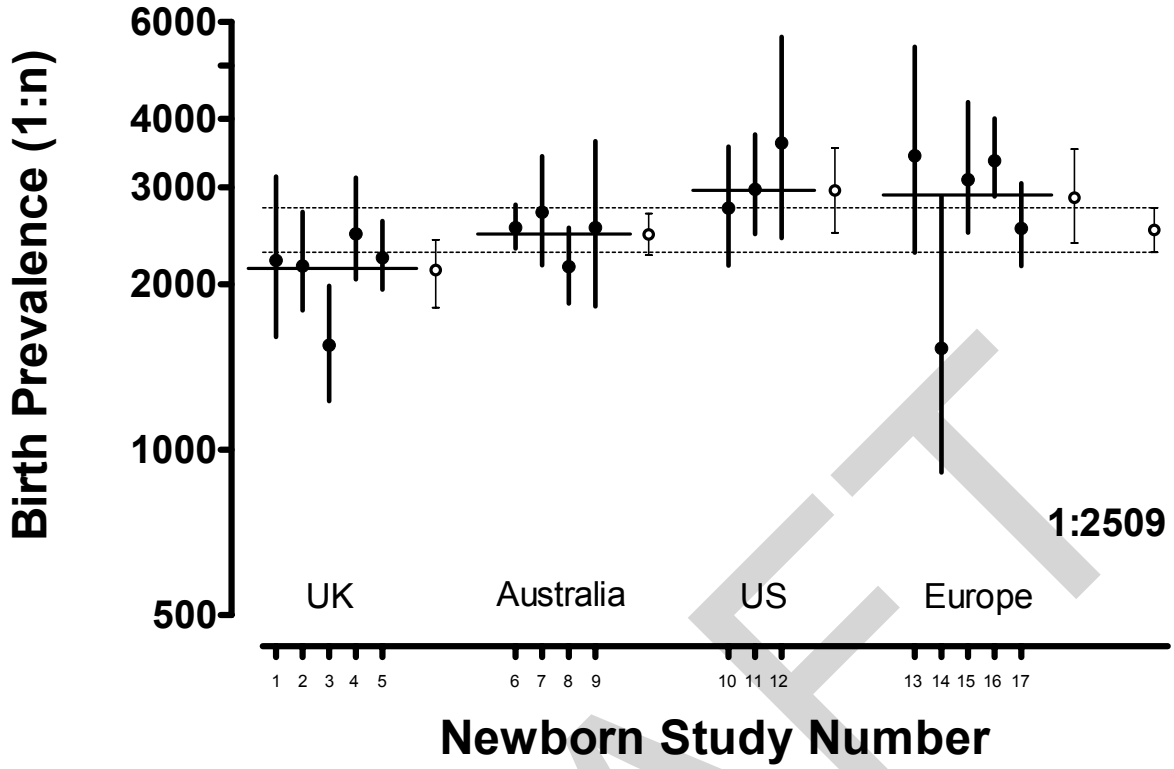

Figure 3-8. Individual and Combined Estimates of Cystic Fibrosis Prevalence in non-Hispanic Caucasians, Derived from Newborn Screening Trials

The following explains how the numbers were derived for each study listed in the table.

United Kingdom

1 *Leeds* – Newborn screening was offered between 1975 and 1994; the more recent time period utilized IRT measurements (Littlewood *et al*., 1995). No adjustments have been made to the numbers.

2 *East Anglia* Newborn screening was offered between 1981 and 1990 using IRT measurements (Green *et al*., 1992). No adjustments have been made to the numbers.

3 *Northern Ireland* – Newborn screening was offered for four years using IRT measurements (Roberts *et al*., 1988). No adjustments have been made to the numbers.

4 *Wales and the Midlands* – Newborn screening for cystic fibrosis was performed on alternative weeks, using a two-step IRT protocol (Ryley *et al.*, 1988). The rate of meconium ileus (8 percent 6/78) was significantly lower than in the corresponding control population (28 percent 19/68), and in the other newborn studies reported (Table 3-21). The authors point out that a non-participating hospital was often used as a referral center for infants with meconium ileus. Thus, the allocation of 19 patients with meconium ileus was not random. The present analysis takes this into account by

allocating six additional cystic fibrosis infants with meconium ileus to the study group. During four of the six years of the study, prenatal diagnosis could have reduced the number of cases identified. An additional nine cases (4/6 * 227,183 / 16,800) are estimated to have been prenatally diagnosed in this population.

5 Trent – Over about six years, newborn screening for cystic fibrosis was offered, using a two-step IRT protocol and, later, a three stage protocol, using IRT and mutation analysis (Pollitt *et al.*, 1997). The entire study was performed during a time when prenatal diagnosis for cystic fibrosis was available. Overall, 26 (437,859/16,800) prenatally diagnosed cases would be expected in this population.

Australia/New Zealand

6 New South Wales – Nearly all of these newborns were screened using a two-step IRT protocol. Subsequently, 200,000 were tested using IRT and mutation analysis (Wilcken *et al.*, 1995). During the time when newborn screening was offered, 24 cases were known to be prenatally diagnosed and terminated, bringing the total cases identified during the 14 years of study to 475. No other corrections are performed.

7 New Zealand – Newborns were screened for cystic fibrosis using a two-step IRT protocol over a seven year time period (Wesley *et al.*, 1989). No modifications are made to the reported numbers. It was assumed that the population was mainly northern European Caucasians.

8 Adelaide – Newborns were screened during one year, using a two-step IRT protocol, beginning in 1989 (Ranieri *et al.,* 1994). For the remaining four years, IRT and mutation analysis was used. During the study, there were 32 prenatal diagnoses and terminations for cystic fibrosis leading to a total of 144 cases identified. No other modifications are made to the reported numbers.

9 Victoria – During the four years of this study, all newborns were tested, using a combination of IRT and mutation analysis (Balnaves *et al.*, 1995). Six prenatal diagnoses were made during that time, leading to a total of 35 cases of cystic fibrosis being identified in the cohort. No other modifications are made to the reported numbers.

United States

10 Colorado – A pilot study was conducted as part of the statewide newborn screening program (Hammond *et al.*, 1991). Screening was by two-step IRT, with sweat testing as the diagnostic test. Compliance and the mixed racial/ethnic nature of the population tested complicate the analysis of prevalence in this study. Only about three-quarters of those with an initially elevated IRT measurement submitted a second sample. In reporting the results of their study, the authors corrected for this non-compliance. However, the study design included follow-up methodology that was designed to identify all missed cases, and three cases of cystic fibrosis were identified in the group with initially elevated IRT measurements that were not re-tested. Because of the study's follow-up, the adjustment (as performed by the authors) appears not to be appropriate and was not performed in our analysis. The study reported that 70 of the 73 cases were

Caucasian and that the associated prevalence was 1:3073 (70:215,110), but it did not distinguish Hispanic from non-Hispanic. Based on 1987 vital statistics, 84.4 percent of the Caucasian population in Colorado was non-Hispanic. Taking this into account reduces the denominator to 181,552 (215,110 * .844). Among Hispanic Caucasians, cystic fibrosis is estimated to have occurred in four newborns. Thus, the corrected non-Hispanic Caucasian prevalence of cystic fibrosis is 1:2750 (66:181,552). The study was performed prior to the availability of prenatal diagnosis.

11 *Wisconsin* - A pilot study was conducted as part of newborn screening in Wisconsin (Gregg *et al.*, 1993; Gregg *et al.*, 1997). Screening was by two-step IRT, with DNA testing incorporated in the later years. A significant proportion of those with elevated screening results did not have the sweat testing completed, but the study's extensive follow-up procedures would have detected any cases occurring in this group. The study reported a rate for Caucasians (1:3431) in the first report (Gregg *et al.*, 1993) but did not stratify by race/ethnicity in the second. Based on the 1989 vital statistics for Wisconsin, 87.8 percent of the population is non-Hispanic Caucasian, 2.2 percent Hispanic, and 10.0 percent Black. Thus, the corrected denominator for non-Hispanic Caucasians is 285,502 (325,173*.878). Three of the observed cases are estimated to have occurred in other racial/ethnic groups. An additional 17 cases (285,502/16,800) would have been detected by newborn screening, had prenatal diagnosis not been available and the present analysis also takes this into account.

12 *Western Pennsylvania* – A pilot newborn screening trial was conducted in several hospitals in the Pittsburgh area (Spence *et al.*, 1993). Screening was by two-step IRT, with DNA testing incorporated in the later years. Even though the population was of mixed race/ethnicity, the study did not take this into account. Based on the 1989 vital statistics for Pittsburgh (percentage of Caucasian and Black) and Pennsylvania (percentage of Hispanic Caucasian), 82.0 percent of the screened population is estimated to be non-Hispanic Caucasian, 2.5 percent Hispanic, 14.5 percent Black and 1 percent Asian. Thus, the corrected denominator for non-Hispanic Caucasians is 86,702, and the corrected number of cases is 19 (one case expected in other racial/ethnic groups has been removed). The study was performed during a time when prenatal diagnosis for cystic fibrosis was widely available, yet this was not taken into account. An additional five cases (86,702/16,800) would have been detected by newborn screening, had prenatal diagnosis not been available and these cases have been included in our estimates.

Europe (w/o United Kingdom)

13 *Normandy, France* – Over a two year time period, newborns were tested using a one-step IRT protocol (Tavert and Duhamel, 1983). Twenty-three infants with cystic fibrosis were identified. No changes to the reported numbers have been made.

14 *Vienna, Austria* – Over a three year time period newborns were screened, using a single IRT measurement (Larsen *et al.,* 1994). Twelve cases of cystic fibrosis were confirmed by sweat testing. The study was performed during a time when prenatal diagnosis was available. One prenatal diagnosis might have occurred in this group (19,992/16,800) and our estimate of prevalence includes this case.

15 Northeastern Italy – Over a three year time period, IRT measurements were used, along with meconium lactase activity on selected samples (Pederzini *et al.*, 1995). During the study, prenatal diagnosis for cystic fibrosis was available, and an estimated nine cases were diagnosed (157,992/16,800). A total of 51 cases of cystic fibrosis are expected in this population.

16 France, Collaborative Study – Over two years, newborn screening was performed in eleven laboratories using a two-step IRT protocol (Dhondt *et al.*, 1993). A total of 122 cases of cystic fibrosis was identified. Another 31 cases are added in the present analysis (513,440/16,800) to account for possible prenatal diagnoses.

17 Brittany, France – Over 10 years, this ongoing screening program identified 118 newborns with cystic fibrosis (Ferec *et al.*, 1995; Scotet *et al.*, 2000). At the beginning, the program used a two-step IRT protocol. In 1993, the program replaced the follow-up IRT measurement with DNA measurements. Prenatal diagnosis identified 18 cases. Thus, 136 cases of cystic fibrosis occurred during the time period covered by the project.

References

Balnaves ME, Bonacquisto L, Francis I, Glazner J, Forrst S. 1995. The impact of newborn screening on cystic fibrosis testing in Victoria, Australia. *J Med Genet* **32**:537-542.

Berlin JA, Laird NM, Sacks HS, Chalmers T. 1989. A comparison of statistical methods for combining event rates from clinical trials. *Stat Med* **8**:141-151.

Chatfield S, Owen G, Ryley HC, Williams J, Alfaham M, Godchild MC, *et al*. 1991. Neonatal screening for cystic fibrosis in Wales and the West Midlands: clinical assessment after five years of screening. *Arch Dis Child* **66**:29-33.

Dhondt JL, Farriaux JP, Briard ML, Boschetti R, Frezal J. 1993. Results of pilot screening activities in the French neonatal screening program – cystic fibrosis, congenital adrenal hyperplasia and sickle cell disease. *Screening* **2**:87-97.

Férec C, Verlingue C, Parent P, Morin JF, Codet JP, Rault G, *et al*. 1995. Neonatal screening for cystic fibrosis: result of a pilot study using both immunoreactive trypsinogen and cystic fibrosis gene mutation analyses. *Hum Genet* **96**:542-548.

Gregg RG, Simantel A, Farrell PM, Koscik R, Kosorok MR, Laxova A, *et al*. 1997. Newborn screening for cystic fibrosis in Wisconsin: comparison of biochemical and molecular methods. *Pediatr* **99**:819-824.

Gregg RG, Wilfond BS, Farrell PM, Laxova A, Hassemer D, Mischler EH. 1993. Application of DNA analysis in a population-screening program for neonatal diagnosis of cystic fibrosis (CF): comparison of screening protocols. *Am J Hum Genet* **52**:616-626.

Green MR, Weaver LT, Heeley AF *et al.* 1993. Cystic fibrosis identified by neonatal screening: incidence, genotype, and early natural history. *Arch Dis Child* **68**:464-467.

Hammond KB, Steven MS, Abman H, Sokol RJ, Accurso FJ. 1991. Efficacy of statewide neonatal screening for cystic fibrosis by assay of trypsinogen concentrations. *N Engl J Med* **325**:769-774.

Larsen J, Campbell S, Faragher EB, Götz M, Eichler I, Waldherr S, *et al*. 1994. Cystic fibrosis screening in neonates – measurement of immunoreactive trypsin and direct genotype analysis for ΔF508 mutation. *Eur J Pediatr* **153**:569-573.

Littlewood JM, LittlewoodAE, McLaughlin S, Shapiro L, Connolly S. 1995. 20 years continuous neonatal screening in one hospital; progress of the 37 patients and their families. 9th North American Cystic Fibrosis Conference, Dallas. *Pediatr Pulmonol* suppl **12**:284.

National Center for Health Statistics: Vital Statistics of the United States 1989, Vol. 1, Natality. DHHS Pub No (PHD) 93-1100. (1993) Public Health Service, Washington, US Government Printing Office.

Pederzini F, Cabrini G, Faraguna D, Giglio L, Mengarda G, Pedrotti D, *et al.* 1995. Neonatal screening for cystic fibrosis using blood trypsin with complementary meconium lactase: an advisable strategy for the population of southern Europe. *Screening* **3**:173-179.

Pollitt RJ, Dalton A, Evans S, Hughes HN, Curtis D. 1997. Neonatal screening for cystic fibrosis in the Trent region (UK): two-step immunoreactive trypsin screening compared with a three-stage protocol with DNA analysis as an intermediate step. *J Med Screen* **4**:23-28.

Ranieri E, Lewis BD, Gerace RL, Ryall RG, Morris CP, Nelson PV, *et al*. 1994. Neonatal screening for cystic fibrosis using immunoreactive trypsinogen and direct gene analysis: four years' experience. *BMJ* **308**:1469-1472.

Roberts G, Stanfield M, Black A, Redmond A. 1988. Screening for cystic fibrosis: a four year regional experience. *Arch Dis Child* **63**:1438-1443.

Scotet V, de Braekeleer M, Roussey M. Rault G, Parent P, Dagorne M, *et al*. 2000. Neonatal screening for cystic fibrosis in Brittany, France: assessment of 10 years' experience and impact on prenatal diagnosis. *Lancet* **356**:789-794.

Spence WC, Paulus-Thomas J, Orenstein DM, Naylor EW. 1993. Neonatal screening for cystic fibrosis: addition of molecular diagnostics to increase specificity. *Biochem Med Metab Bio* **49**:200-211.

Travert G, Duhamel JF. 1983. Depistage neonatal systematique de la mucoviscidose par dosage de la trypsine immunoreactive sanguine. *Arch Fr Pediatr* **40**:295-298.

Wesley AW, Smith PA, Elliott RB. 1989. Experience with neonatal screening for cystic fibrosis in New Zealand using measurement of immunoreactive trypsinogen. *Aust Paediatr J* **25**:151-155.

Wilcken B, Wiley V, Sherry G, Bayliss U. 1995 Neonatal screening for cystic fibrosis: A comparison of two strategies for case detection in 1.2 million babies. *J Pediatr* **127**:965-970.

Cystic fibrosis prevalence: Population-based registries

Table 3-22 lists the population-based cystic fibrosis registries which serve as the source for the present analysis. The present summary includes only reports from registries that summarize data from the 1970s or later. The three registries in Table 3-22 use multiple sources of ascertainment over a relatively long time period (up to ten years) to capture nearly all clinically defined cases. An advantage of studies that include only births prior to 1989 is that they will not be influenced by prenatal diagnosis. Data from each of the registries will be explained in more detail in the following sections.

Table 3-22. Cystic Fibrosis Registries Serving as Data Sources for the Present Analysis

Study Number	Location	Years	Race / Ethnicity	Years Followed
1	United Kingdom	1978-87	NHC	>10
2	Canada	1970-79	Mixed	>10
3	United States	1990-1992	Mixed	2

NHC – non-Hispanic Caucasians; Mixed – NHC and other racial/ethnic groups

Table 3-23 shows the numbers of non-Hispanic Caucasians included in cohorts from each registry, along with the number of cases identified. In several instances, the numbers have been adjusted for various ascertainment biases or for mixed race/ethnicity. These adjustments are described in detail in the following sections. The overall prevalence estimate of 1:2499 is computed using a random-effects model (Berlin *et al.,* 1989**)**. Heterogeneity is detected between the three prevalence estimates ($\chi^2 = 4.1$, $p = 0.04$); the Canadian estimate is somewhat lower than those for the United Kingdom and United States. Figure 3-9 graphically displays the adjusted birth prevalences for non-Hispanic Caucasians from these three Registries.

Table 3-23. Birth Prevalence of Cystic Fibrosis for Non-Hispanic Caucasians Derived from Population-Based Registries

Study Number	Total NHC	CF Cases	Birth Prevalence	95% Confidence Interval
1	7,360,000	3,046	1:2416	2333-2507
2	3,041,510	1,168	1:2604	2462-2717
3	7,675,221	3,086[1]	1:2487	2396-2583
All	18,076,731	7,300	1:2499	2371-2633

[1] Corrected for reported under-coverage of 17 percent and under-diagnosis of 11 percent.

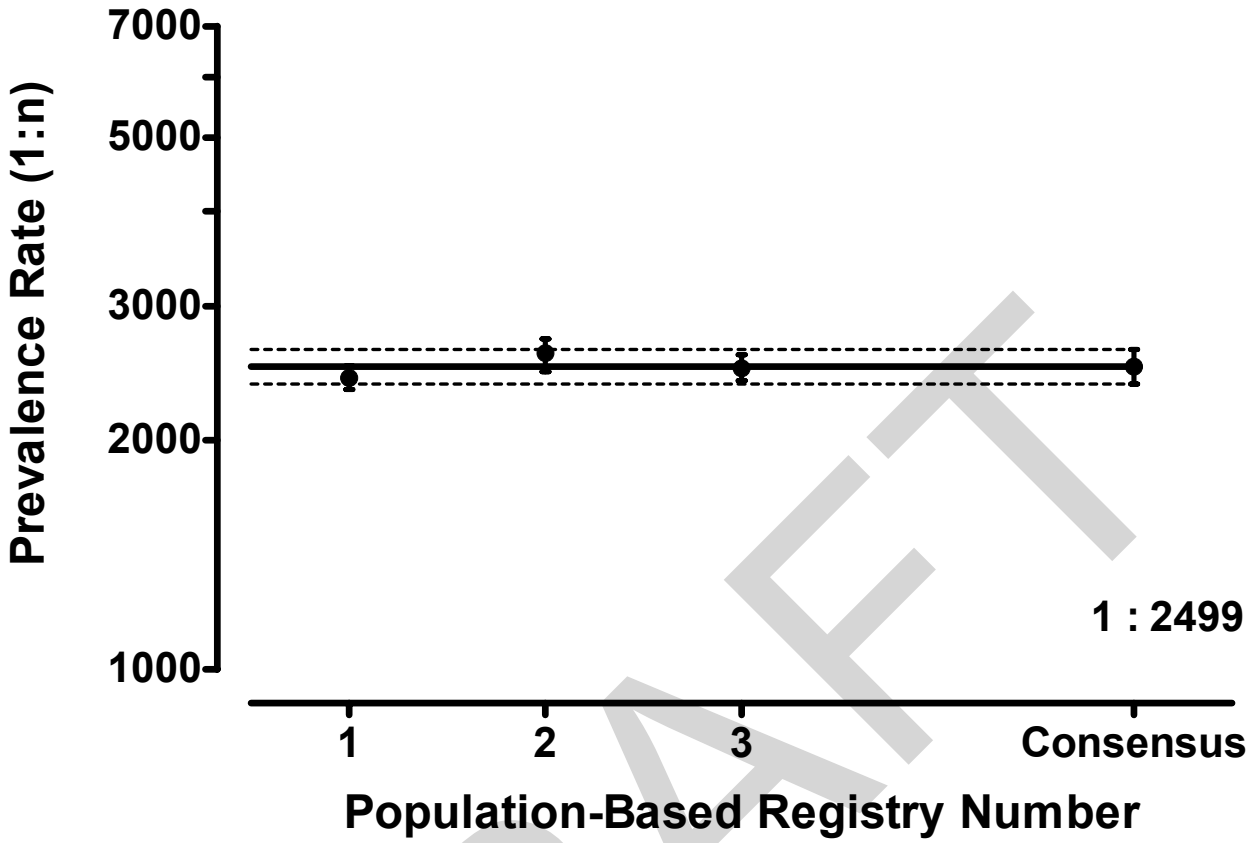

Figure 3-9. Estimated birth prevalence of cystic fibrosis for non-Hispanic Caucasians according to three registries

United Kingdom – The United Kingdom has prospectively collected information on cystic fibrosis patients since 1978 (Dodge *et al.,* 1993; Dodge *et al.,* 1997). A retrospective calculation of rates between 1968 and 1978 is not included, but these are similar to the later time period. According to the most recent report (Dodge *et al.,* 1997) the birth prevalence estimates are reliable only through 1987, because of late diagnoses. The 1978 through 1987 cohort has been followed for at least 10 years. The analysis treats the entire population as non-Hispanic Caucasian, although this is known not to be the case. For that reason, the listed birth prevalence estimate should be considered an underestimate. No important differences were found by region within the United Kingdom. The reported data have not been modified.

Canada – A Canadian registry has monitored cystic fibrosis occurrence prospectively since 1970 (Corey and Farewell, 1997). The data from 1970-1979 are considered to be 96 percent complete as of 1989. The most recent birth cohort has been followed for at least 10 years. In 1970, 10 percent of the Canadian population was non-Caucasian, rising to 20 percent in 1980. To account for this, the present analysis reduces the total cohort by 15 percent. Ninety-eight percent of the cases were from within the Caucasian population, but no estimate of numbers of Hispanic Caucasians is provided. Thus, the listed birth prevalence estimate should be considered an

underestimate. A recent report from Nova Scotia included data through 2000 (Chong *et al.,* 2000). After correction for delayed diagnosis, the estimate was 1:2436 (95 percent CI 2028 to 3049).

United States – The Cystic Fibrosis Foundation has maintained a National CF Patient Registry for all cases attending CF Foundation –accredited Centers. This does not include patients seen at health maintenance organizations, by private practitioners or in the armed forces. In an unpublished study, it was estimated that 83 percent of the cases could be ascertained (Hamoush *et al.*, 1998). The study reported the average yearly birth prevalence between 1990 and 1992, so the numbers (from Table 2 of that study) have been multiplied by three. The study based its cohort on all births in the United States, using statistics from the National Center for Health Statistics, (3,201,678 Caucasian births occurred in 1992). However, 643,271 Hispanic births also occurred; nearly all of these (96 percent) being Caucasian, as well. Hispanic births have been removed in the present analysis, along with the associated 58 cases of cystic fibrosis occurring annually. No estimate was provided in the registry as to the extent of under-ascertainment, due to the relatively short follow-up time period of two years. However, a recent report (Kosorok *et al.,* 1996) studied the same registry data for the years 1989-1991 and statistically corrected for under-ascertainment due to delayed diagnosis (similar to the methodology used by Chong *et al.,* 2000). They found a prevalence in all Caucasians of 1:2826 (after correcting for the 83 percent coverage noted above) compared to 1:3200 using the current report (Hamosh *et al.,* 1998). This suggests that 12 percent of cases were not yet identified in the cohort. This is consistent with the CF Foundation's own finding that about 11 percent of cases are identified between 3 and 10 years of age (CF Annual Report, 2000, Figure 3). The Canadian report (Corey and Farewell, 1996) found 14 percent, but that was during the 1970's and 1980's when diagnosis might have been more delayed than current practice. To account for the relatively short period of follow-up, the number of identified cases has been corrected by 11 percent.

Possible ascertainment bias

It is possible that the newborn screening trials should also be corrected for short-term follow-up. However, it is also possible that IRT measurements will identify such a high percentage of cases prior to symptoms that only a small number of missed cases are subject to this bias. If so, this bias is unlikely to have much of an impact on the newborn screening trials.

References

Berlin JA, Laird NM, Sacks HS, Chalmers T. 1989. A comparison of statistical methods for combining event rates from clinical trials. *Stat Med* **8**:141-151.

Brunecky Z. 1972. The incidence and genetics of cystic fibrosis. *J Med Genet* **9**:33-37.

Chong MY, Hamilton DC, Cole DEC. 2000. Retrospective estimation of the birth prevalence for delayed onset disorders: application to cystic fibrosis in Nova Scotia. *Stat Med* **19**:743-751.

Corey M, Farewell V. 1996. Determinates of mortality for cystic fibrosis in Canada, 1970-1989. *Am J Epidemol* **143**:1007-1017.

Dodge JA, Morison S, Lewis PA, Coles EC, Geddes D, Russell G, *et al*. 1993. Cystic fibrosis in the United Kingdom, 1968-1988: incidence, population and survival. *Paediatr Perinat Epidemol* **7**:157-166.

Dodge JA, Morison S, Lewis PA, Coles EC, Geddes D, Russell G, *et al*. 1997. Incidence, population , and survival of cystic fibrosis in the UK, 1968-95. *Arch Dis Child* **77**:493-496.

Hamosh A, FitzSimmons SC, Macek M, Knowles MR, Rosenstein BJ, Cutting GR. 1998. Comparison of the clinical manifestations of cystic fibrosis in black and white patients. *J Pediatr* **132**:255-259.

Kosorok MR, Wei WH, Farrell PM. 1996. The incidence of cystic fibrosis. *Stat Med* **15**:449-462.

Selander P. 1962. The frequency of cystic fibrosis in the Pancreas in Sweden. *Acta Paediatr* **51**:65-67.

ten Kate LP. 1977. Cystic fibrosis in the Netherlands. *Int J Epidemol* **6**:23-34.

Prevalence in racial/ethnic groups other than non-Hispanic Caucasians

Ashkenazi Jewish

Data are available to estimate the birth prevalence of cystic fibrosis in the Ashkenazi Jewish population from five studies reporting carrier frequencies and one study using a population-based registry in Israel. Table 3-24 shows the estimated birth prevalence after selected adjustments have been performed. A short description of each study is included after the table. Overall, the birth prevalence is 1:2271, (95% confidence interval 1:1793 to 1:2876), but there is a wide range in estimates from a high of 1:1639 to a low of 1:3123. This heterogeneity is significant ($\chi^2 >$ 400, $p < 0.001$), but no explanation for this is apparent. The two lowest estimates are from the United States. Figure 3-10 shows the same data in graphic form.

Table 3-24. Birth Prevalence of Cystic Fibrosis for the Ashkenazi Jewish Population

Study	Type	Number	Positive	Prevalence [1]	95% CI
Texas	Carrier	1364	62	1:1639	(1041-2685)
New York	Carrier	595	25	1:1907	(927-4300)
Israel 2001	Carrier	6858	273	1:2133	(1721-2713)
Israel 1992	Carrier	424	13	1:3069	(1173-9426)
Israel 1995	Population	207,111	63	1:3123	(2441-4063)
All Studies				1:2271	(1793-2876)

[1] After adjustment of carrier studies for the proportion of mutations detected and population studies for length of follow-up.

1 Texas 1996 DeMarchi and colleagues performed genetic testing for cystic fibrosis in combination with testing for Tay-Sachs and Gaucher disease among young Ashkenazi Jewish adults. Testing was for the five most common mutations (W1282X, delF508, G542X, 3849+10C>T, and N1303K). According to Table 3-10 earlier in this section, 92 percent of the mutations are detectable, using this panel. This is taken into account in the prevalence estimate provided in the table.

2 New York 1998 Kronn and colleagues performed prenatal genetic screening for three genetic disorders associated with Jewish ethnicity, including cystic fibrosis. Testing was for the same five mutations as several other studies (W1282X, delF508, G542X, 3849+10C>T, and N1303K). According to Table 3-10 earlier in this section, 92 percent of the mutations are detectable using this panel. This has been taken into account in the prevalence estimate provided in the table. One patient with an atypical presentation was diagnosed as having cystic fibrosis and was not included in the analysis. No patients with a family history were included.

3 Israel 2001 Orgad and colleagues studied 6,850 Ashkenazi Jewish individuals. The mutation results were stratified by the five most common mutations (W1282X, delF508, G542X, 3849+10C>T, and N1303K) and four others (D1152H, 405+1G>A, W1089X and S549R). The

analysis shown in Table 3-17 uses only the results of the five most common mutations, since they were tested for in all individuals. Once again, the estimates take into account that these mutations represent 92 percent of the total mutations. This study reports an unexpectedly high rate of occurrence of the D1152H mutation: 1 in 114 individuals tested. This mutation is part of the secondary panel and is not included in the prevalence calculation. This implies that about 18 percent of the CF mutations in affected individuals should be D1152H. However, the 1995 study by Kerem and colleagues tested for this mutation in 261 Ashkenazi Jewish individuals with cystic fibrosis and did not find a single occurrence (although 2 D1152H mutations were identified among 105 affected Jewish individuals who were non-Ashkenazi). This strongly suggests that the D1152H mutation has a low penetrance and that its inclusion in a screening panel will yield information that is difficult to interpret, at least for Ashkenazi Jewish individuals.

4 Israel 1992 Abeliovich and colleagues tested 848 chromosomes (equivalent to 424 individuals) for three mutations (W1282X, delF508 and G542X) and found 13 heterozygotes. According to Table 3-10 earlier in this section, 85 percent of the mutations are detectable using this panel. This has been taken into account in the prevalence estimate provided in the table.

5 Israel 1995 Kerem and colleagues identified all cases of cystic fibrosis born between 1981 and 1987 in Israel. Given the follow-up time of 5 years, it is possible that a small percentage of cases was not yet diagnosed. Information presented earlier showed that between 11 and 14 percent of cystic fibrosis cases are not identified until 3 to 10 years after birth. To take this into account, we assume a 5 percent under-ascertainment. Little information was provided in the report to document the extent to which all cases were identified, and it is possible, therefore, that this estimate is low.

Figure 3-10. Estimated Birth Prevalence of Cystic Fibrosis in the Ashkenazi Jewish Population According to the 5 Published Studies in Table 3-17

References

Abeliovich D, Lavon IP, Lerer I, Cohen T, Springer C, Avital A, Cutting GR. 1992. Screening for five mutations detects 97% of cystic fibrosis (CF) chromosomes and predicts a carrier frequency of 1:29 in the Jewish Ashkenazi population. *Am J Hum Genet* **41**:951-956.

DeMarchi JM, Caskey CT, Richards CS. 1996. Population-specific screening by mutation analysis for diseases frequent in Ashkenazi Jews. *Hum Mutat* **8**:116-125.

Kerem E, Kalman YM, Yahav Y, Shoshani T, Abeliovich D, Szeinberg A, *et al*. 1995. Highly variable incidence of cystic fibrosis and different mutation distribution among different Jewish ethnic groups in Israel. *Hum Genet* **96**:193-197.

Kromn D, Jansen V, Ostrer H. 1998. Carrier screening for cystic fibrosis, Gaucher disease, and Tay-Sachs disease in the Ashkenazi Jewish population. *Arch Intern Med* **158**:777-781.

Orgad S, Neumann S, Loewenthal R, Netanelov-Shapira I, Gazit E. 2001. Prevalence of cystic fibrosis mutation in Israel Jews. *Genet Test* **5**:47-52.

Hispanic Caucasians in the United States

Data are available to estimate the birth prevalence of cystic fibrosis in Hispanic Caucasians from three studies reporting carrier frequencies and one study utilizing a population-based registry in the United States. Table 3-25 shows the estimated birth prevalence after selected adjustments have been performed. A short description of each study is included after the table. Overall, the birth prevalence is 1:13,535, (95% confidence interval 1:6,800 to 1:27,000), but there is a wide range in estimates from a high of 1:2430 to a low of 1:27,000. This heterogeneity is significant ($\chi^2 > 48$, $p < 0.001$), but no explanation is apparent. Two relatively large studies reporting the carrier rate in Hispanic women in the US are consistent with birth prevalences much lower than that reported by the CF Foundation survey. The birth prevalence estimate of 1:9,200 reported by the CF Foundation corresponds to a carrier rate of 1:48. Thus, the three screening trials would be expected to identify about 28 carriers among the 2,171 women (63 percent * 2171/48). However, only 20 were actually found. Figure 3-11 shows the birth prevalence data for this population group in graphic form.

Table 3-25. Birth Prevalence of Cystic Fibrosis for Hispanic Caucasians

Study	Type	Number	Positive	Prevalence [1]	95% CI
Rochester NY	Carrier	78	2	1: 2430	(328-39,000)
CF Foundation	Population	1,929,813	174	1: 9,200	(8,050-10,600)
N California	Carrier	1,053	10	1:20,000	(7,300-62,500)
S California	Carrier	1,040	8	1:27,000	(9,100-98,000)
All Studies				1:13,535	(6,800-27,000)

[1] After adjustment of carrier studies for the proportion of mutations detected and population studies for incomplete ascertainment.

1 Rochester NY Loader and colleagues (1996) performed prenatal screening for cystic fibrosis; 4,879 women were tested, including 78 Hispanic women. Testing was for six common mutations (delF508, G542X, G551D, R553X, W1282X, and N1303K). According to Table 3-6 shown earlier in this section, this panel detects 63 percent of the mutations. This is taken into account in the prevalence estimate provided in Table 3-18.

2 CF Foundation Hamosh and colleagues (1998) relied on data from the Cystic Fibrosis Foundation Patient Database to estimate the prevalence of cystic fibrosis among self-declared Hispanic individuals in the United States. The study found an average of 58 cases in Hispanic individuals per year between 1990 and 1992. The National Center for Health Statistics reported 643,271 Hispanic births each year during the same time period, yielding an estimated prevalence of 1:9,200 after correction for incomplete ascertainment (that study used a multiplication factor of 1.21).

3. N California Witt and colleagues (1996) performed prenatal screening for cystic fibrosis; 1,053 Hispanic women were enrolled. Of these, 306 were tested for the six common mutations

(accounting for 63 percent of all mutations), and 747 were tested for 12 mutations (accounting for 67 percent of all mutations). Ten carrier women were identified.

4. S California Grody and colleagues (1997) performed prenatal screening for cystic fibrosis; 1,040 Hispanic women were enrolled (two-thirds classified themselves as Mexican Hispanics). They were tested for the six common mutations. Eight carrier women were identified.

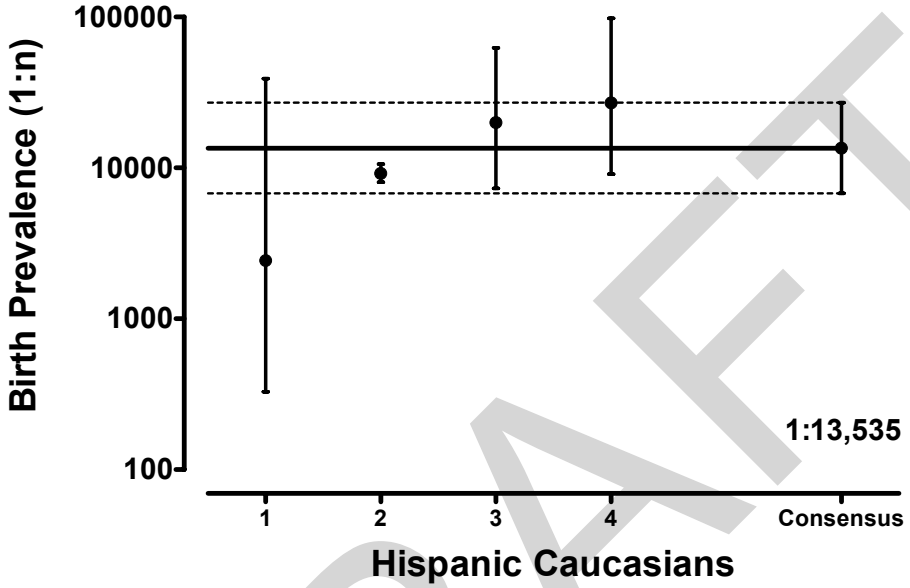

Figure 3-11. Estimated Birth Prevalence of Cystic Fibrosis in Hispanic Caucasians According to the 4 Published Studies in Table 3-25

References

Grody WW, Dunkel-Schetter C, Tatsugawa ZH, Fox MA, Fang CY, Cantor RM, *et al*. 1997. PCR-based screening for cystic fibrosis carrier mutations in an ethnically diverse pregnant population. *Am J Hum Genet* **60**:935-947.

Hamosh A, FitzSimmons SC, Macek M, Knowles MR, Rosenstein BJ, Cutting GR. 1998. Comparison of the clinical manifestations of cystic fibrosis in black and white patients. *J Pediatr* **132**:255-259.

Loader S, Caldwell P, Kozyra A, Levenkron JC, Boehm CF, Kazazian HH, Rowley PT. 1996. Cystic fibrosis carrier population screening in the primary care setting. *Am J Hum Genet* **59**:234-237.

Witt DR, Schaefer C, Hallam P, Wi S, Blumberg B, Fishbach A, *et al*. 1996. Cystic fibrosis heterozygote screening in 5,161 pregnant women. *Am J Hum Genet* **58**:823-825.

African Americans

Data are available to estimate the birth prevalence of cystic fibrosis in African Americans from two studies reporting carrier frequencies and three studies uses population-based registries. Table 3-26 shows the estimated birth prevalence after selected adjustments have been performed. A short description of each study is included after the table. Overall, the birth prevalence is 1:15,057, (95% confidence interval 1:14,800 to 1:15,300). The two population-based estimates are similar and indicate a carrier rate of about 1:61. The test panels used in the two carrier studies identify only about 48 percent of the mutations. Therefore, three carriers should be found in the 369 African Americans tested in the two carrier studies shown in Table 3-26. None were identified.

Table 3-26. Birth Prevalence of Cystic Fibrosis for African Americans

Study	Type	Number	Positive	Prevalence [1]	95% CI
CF Foundation	Population	2,020,899	111	1:15,050	(12,900-18,200)
Washington, DC, 74	Population	136,267	8	1:17,033	(8,600-39,500)
Washington, DC, 89	Population	86,162	5	1:17,232	(7,400-53,00)
Rochester NY	Carrier	100	0	undefined	(3,052->99,999)
S California	Carrier	269	0	undefined	(22,000->99,999)
All Studies				1:15,057	(14,800-15,300)

[1] After adjustment of the CF Foundation population studies for incomplete ascertainment.

1 CF Foundation Hamosh and colleagues (1998) used data from the Cystic Fibrosis Foundation Patient Database to estimate the prevalence of cystic fibrosis among self-declared African Americans. The study found an average of 37 cases per year between 1990 and 1992. The National Center for Health Statistics reported 673,633 African American births each year during the same time period, yielding an estimated prevalence of 1:15,050 after correction for incomplete ascertainment (that study utilized a multiplication factor of 1.21).

2 Washington, DC Kulczycki and colleagues (1974) surveyed the District of Columbia between 1962 and 1971 for cases of cystic fibrosis in African Americans. They identified eight cases among the 136,267 births during that time period.

3 Washington DC Prapphal and colleagues (1989) surveyed the District of Columbia between 1975 and 1985 for cases of cystic fibrosis in African Americans. They identified five such individuals among the 86,162 births during that time period.

4 Rochester NY Loader and colleagues (1996) performed prenatal screening for cystic fibrosis, including 100 African American women. Testing was for six common mutations (delF508, G542X, G551D, R553X, W1282X, and N1303K). According to Table 3-6 shown earlier in this section, 48 percent of the mutations are detectable, using this panel. However, since no cystic fibrosis mutations were identified, no estimate of prevalence can be made.

5 S California Grody and colleagues (1997) performed prenatal screening for cystic fibrosis, including 269 African American women. They were tested for the six common mutations that would be expected to identify 48 percent of the mutations in this population. Again, no carriers were identified.

References

Grody WW, Dunkel-Schetter C, Tatsugawa ZH, Fox MA, Fang CY, Cantor RM, *et al*. 1997. PCR-based screening for cystic fibrosis carrier mutations in an ethnically diverse pregnant population. *Am J Hum Genet* **60**:935-947.

Hamosh A, FitzSimmons SC, Macek M, Knowles MR, Rosenstein BJ, Cutting GR. 1998. Comparison of the clinical manifestations of cystic fibrosis in black and white patients. *J Pediatr* **132**:255-259.

Loader S, Caldwell P, Kozyra A, Levenkron JC, Boehm CF, Kazazian HH, Rowley PT. 1996. Cystic fibrosis carrier population screening in the primary care setting. *Am J Hum Genet* **59**:234-237.

Kulczycki LL, Schauf V. 1974. Cystic fibrosis in Blacks in Washington, DC. *Am J Dis Child* **127**:64-67.

Prapphal N, Fitzpatrick SB, Getson P, Fink R, O'Donnell R, Chaney H. 1989. Cystic fibrosis in blacks in Washington, DC: fifteen years' experience. *J Natl Med Assoc* **81**:263-267.

Asian Americans

There are two published estimates of cystic fibrosis in Asians in their native lands. One found a prevalence of 1:90,000 in Asian Hawaiians (Wright *et al*., 1968). Another found a prevalence of 1:320,000 in Japan between 1969 and 1980 (Imaizumi, 1995). As of 1998, the literature contained only 40 detailed reports of cystic fibrosis in Asians, and many of the cases were of mixed heritage (Suwanjutha *et al*., 1998). As of that time, all instances of delF508 occurring in 'Asian' cystic fibrosis patients could be traced to documented Caucasian admixture. Thus, the prevalence for cystic fibrosis among Asians could be 1:100,000, or even lower. Another report (Curtis, 1993) tested 400 individuals from India and 43 'Orientals' for delF508, G551D, R553X and S549N and found no heterozygotes.

On the other hand, it is likely that individuals might be classified as 'Asian' in the United States, even if some Caucasian admixture had occurred. Using the Cystic Fibrosis Foundation Patient Database, one study has estimated the prevalence of cystic fibrosis among self-declared Asian individuals in the United States (Hamosh *et al*., 1998). The study found an average of 4 cases in Asian Americans per year between 1990 and 1992. The National Center for Health Statistics reported 140,250 Asian American births each year during the same time period, yielding an estimated prevalence of 1:31,000. This is significantly higher than the two studies quoted earlier, and this difference is probably due to admixture; one participating center reported that 'of the five Asian-American patients with CF, four had one white parent'.

References

Curtis A, Richardson RJ, Boohene J, Jackson A, Nelson R, Bhattacharya SS. 1993. Absence of cystic fibrosis mutations in a large Asian population samples and occurrence of a homozygous S549N mutations in an inbred Pakistani family. *J Med Genet* **30**:164-166.

Hamosh A, FitzSimmons SC, Macek M, Knowles MR, Rosenstein BJ, Cutting GR. 1998. Comparison of the clinical manifestations of cystic fibrosis in black and white patients. *J Pediatr* **132**:255-259.

Imaizumi A. 1995. Incidence and mortality rates of cytsic fibrosis in Japan, 1969-1992. *Am J Med Genet* **58**:151-158.

Suwanjutha S, Huang NN, Wattanasirichaigoon D, Sura T, Harris A, Macek M. 1998. Case report of a Thai male cystic fibrosis patient with the 1898+1G>T splicing mutation in the CFTR gene: A review of east Asian cases. Mutations in brief no. 196. Online. *Hum Mutat* **12**:361.

Wright SW, Morton NE. 1968. Genetic studies on cystic fibrosis in Hawaii. *Am J Hum Genet* **20**:157-169.

Question 22: Has the test been adequately validated on all populations to which it may be offered?

Summary

- The analytic performance for selected cystic fibrosis mutations is expected be consistent regardless of the race/ethnicity of the population being tested.
- It is possible, however, that rare unknown polymorphisms (that could cause false positive results) might vary by race/ethnicity

The DNA testing utilized for prenatal screening is aimed at identifying specific mutations that cause cystic fibrosis. The test is designed to identify these mutations in any DNA sample regardless of the characteristics of the individual being tested (e.g., race or ethnicity). Although the prevalence of cystic fibrosis and the mix of mutations responsible for the disorder may vary by race, the test should reliably identify the target mutation. One exception to this might occur if the presence and/or frequency of unknown polymorphisms would vary by race/ethnicity (or some other factor). In reality, however, it would be difficult for laboratories to thoroughly examine this possibility in all populations to which testing may be offered.

Gap in Knowledge: Polymorphisms by race/ethnicity.
Variation in polymorphism frequency by race/ethnicity has not been well described in the literature. Laboratories should make efforts to report in the literature all polymorphisms in the context of the racial/ethnic background being tested.

Clinical Validity

Question 23: What are the positive and negative predictive values?

Summary

- The positive predictive value is dependent on the birth prevalence, the analytic sensitivity, the clinical specificity and the screening model employed. It is not strongly dependent on the proportion of detectable mutations.
- The least well defined of these factors is the impact of confirmatory testing on the analytic specificity and its influence on clinical specificity.
- Using reasonable estimates for these factors, positive predictive values are at least 99 percent and probably over 99.9 percent in non-Hispanic Caucasians and Ashkenazi Jewish individuals. In other words, 10 in 1,000 or fewer screen positive couples might be incorrectly classified.
- Positive predictive values for diagnostic studies in the fetus are likely to be very high, but few confirmatory data are available.

- The negative predictive value is dependent on the screening model used, the combination of test results in the couple, birth prevalence, and the analytic and clinical sensitivity. It is not strongly dependent on the analytic or clinical specificity.
- Because cystic fibrosis is relatively rare, negative predictive values are expected to be very high, regardless of small variations in test performance.
- Using reasonable estimates for these factors, certain types of negative test results (one partner is positive and the other is negative) actually increase the risk for having an affected fetus over the background risk in the population, even though there are no additional tests to further reduce that risk. This is especially true when the mutation detection rate is low.
- In other test combinations for the couple, the risk is reduced below the birth prevalence by between 2 and 20-fold

Positive predictive values

There are three possible definitions for positive predictive value. All are based on the principles shown earlier in this section (Question 18, Table 3-2).

- Given screen positive couples, what proportion are actually carrier couples
- Given screen positive couples, what proportion of their pregnancies will be affected
- Given a positive fetal diagnostic test, what proportion of fetuses would eventually develop the cystic fibrosis phenotype

Each will be discussed in more detail in the following sections.

The positive predictive values for being a carrier couple given that both partners have an identified mutation are dependent on the

- birth prevalence of cystic fibrosis (varies from 1:2,500 to 1:31,000 depending on race/ethnicity)
- proportion of cystic fibrosis mutations identified (varies from 40 to 95 percent depending on race/ethnicity)
- analytic sensitivity (expected to be constant at about 97.9 percent)

- analytic specificity and the subsequent performance of confirmatory testing to identify false positive results. This rate is not well established. For the purposes of the table, the final analytic specificity (after all confirmatory testing has been performed) will be modeled at rates between 99,900 and 99,999 per 100,000 tests (i.e., false positive rates between 1 and 100 per 100,000).
- Screening model used. Table 3-27 is appropriate for the one-step (sequential) and two-step (couple) models only. The expanded two-step (concurrent) model will have approximately twice the number of false positive couples identified because it identifies twice the number of couples in which one is a true positive (since all samples are tested). To compute the positive predictive value for the concurrent model, divide the odds by 2 and recompute the positive predictive value. For example, the first row in Table 3-27 shows a positive predictive value of 97.1 percent (odds of 33:1 or 33/34). For the concurrent model, the corresponding number would be 94.3 percent (odds of 33/2:1 or 16.5/17.5).

Taking the above factors into account, Table 3-27 shows the corresponding positive predictive values. These values do not vary much with the changing proportions of mutations detected within the range of values provided (Column 2). The positive predictive values are, however, strongly dependent on both the prevalence and the false positive rate. In viewing the positive predictive values, it is important to recognize that the number of couples with positive screening results is initially quite low; 1 in 625, 1 in 2,500 and 1 in 7500, respectively, for the three prevalences shown in the table. Prenatal testing identifies a group with risks several thousand times higher.

Table 3-27. Estimates of the Positive Predictive Value for Being a Carrier Couple when the One-step (Sequential) or Two-step (Couple) Screening Models are Employed at Three Birth Prevalences and Three False Positive Rates

Birth Prevalence	Mutations Detected (%)	False Positive Rate (per 100,000)	Positive Predictive Value (%)	Positive Predictive Value Odds (n:1)
1:2,500	75 – 95	100	97.1	33
		10	99.7	340
		1	99.9	3,400
1:10,000	60 – 80	100	92.9	13
		10	99.3	140
		1	99.9	1,400
1:30,000	40 – 60	100	82.9	4.8
		10	98.2	56
		1	99.8	570

Actual data to confirm this modeling are scarce. A preliminary estimate of the positive predictive value can be made, based on information collected as part of ongoing prenatal cystic fibrosis diagnostic testing. As described in an earlier section, a major prenatal diagnostic referral

laboratory in the United States requires that carrier couples submit new blood samples and the parental genotypes along with the amniotic fluid. The referral laboratory has documented false positive carrier classification on more than one occasion (Heim R, personal communication, 2001). This preliminary observation suggests that the false positive rate (after confirmatory testing is completed) is likely to be between 1 and 10 per 100,000 couples tested.

The positive predictive values for having an affected fetus given a carrier couple are dependent on the proportion of carrier couples correctly identified (positive predictive value for carrier couples from Table 3-27). Were all carrier couples to be correctly classified, their risk for an affected fetus would be 1 in 4 (odds 1:3), assuming that all mutations are highly penetrant (see Questions 20 and 24 for a further discussion). The risks change only slightly when the positive predictive value for carrier couples is reduced to as low as 95 percent (risk of 23.8 percent, odds 1:3.2). Were the positive predictive value for carrier couples to be as low as 80 percent, the risk would be appreciably lower (20 percent, odds 1:4). Given the likely positive predictive values for carrier couples, the approximate risk of 25 percent (odds of 1:3) is appropriate for counseling purposes when the penetrance of the mutations identified are known to be high.

The positive predictive values for having an offspring with the cystic fibrosis phenotype given a positive fetal diagnostic test are mainly dependent on the genotype/phenotype relationship and the error rate for diagnostic testing. Error could occur during fetal diagnostic testing because of maternal cell contamination. For that reason, it is important that laboratories performing fetal diagnostic testing collect parental genotypes. Currently, there is little information available concerning the reliability of cystic fibrosis testing of fetal cells. Nearly all fetuses with two identifiable mutations will eventually develop the cystic fibrosis phenotype, but a small proportion will be less severely affected. The relationship between genotype and phenotype (Question 24) and the impact of the environment and other genes on the phenotype (Question 25) are discussed in more detail in the following sections. Also, a few of the uncommon mutations are often not always associated with the classic phenotype. This is discussed in another section (Question 20).

Gap in Knowledge: The Performance of Cystic Fibrosis Mutation Analysis as a Prenatal Diagnostic Test

The analytic sensitivity and specificity of cystic fibrosis testing in fetal cells obtained by amniocentesis or chorionic villus sampling is not well documented. Maternal cell contamination might rarely contribute to false positive results, especially if the result is based on uncultured cells.

Negative predictive values

The negative predictive value is defined in this section as the probability of a couple with a negative test results not having a child with cystic fibrosis and is based on the principles shown earlier in this section (Question 18, Table 3-2). As discussed earlier, one of three screening models could be employed, two-step (or sequential), one-step (or couple) and an expanded one-step (concurrent). In the two step model, there are two types of negative results: the woman tests negative and the partner is not tested (N/NT), and the woman tests positive and the partner tests negative (P/N). In the one-step model, all couples are reported as negative, unless both partners are identified as carriers (CNP – couple not positive). In the expanded one-step model, all couples' samples are tested and two types of negative test results are possible; one partner tests positive and the other negative (P/N), and both partners test negative (N/N). When necessary, the following section will provide negative predictive values stratified by model and couple test results.

The negative predictive values are dependent on

- the screening model and combination of test results in the couple
- the birth prevalence of cystic fibrosis (varies from about 1:2,500 to 1:31,000 depending on race/ethnicity)
- the proportion of cystic fibrosis mutations detected (varies from about 40 to 95 percent depending on race/ethnicity)
- analytic sensitivity (expected to be constant at about 97.9 percent)

Analytic specificity has little impact on the negative predictive value, even when confirmatory testing to identify false positive results is taken into account. For that reason, it is not included in the present calculations.

Table 3-28 shows negative predictive values under a variety of circumstances. These values are strongly dependent on the screening model and combination of the couple's test results, the prevalence of cystic fibrosis and the proportion of mutations detected. In viewing the negative predictive values, it is important to recognize that nearly all couples do not include two carrier. When the birth prevalence is relatively high (i.e., 1:2,500), 624 of every 625 couples do not include two carriers. Therefore, those couples will not have a child with cystic fibrosis. According to the table, the higher the mutation detection rate, the lower the risk in those couples with negative test results. This is because higher mutation detection rates will be associated with the identification of more true carrier couples, and those couples are not included in this table.

Not addressed here are the more complicated scenarios where the partners are of differing ethnic/racial backgrounds. When this occurs, the negative predictive values will differ from those in the table and are even dependent on which partner is tested first (unless the expanded one-step (concurrent) model is employed).

Table 3-28. Negative Predictive Value by Test Model and the Couple's Test Result at Three Birth Prevalences and Three Proportions of Mutations Identified

CF Birth Prevalence	Proportion Of Mutations Identified (%)	Negative Predictive Value (Odds of 1:n)[1]				
		Two-Step		One-Step	Expanded One-Step	
		N/NT	P/N	CNP	N/N	P/N
1:2,500	95	99.99 (34,000)	99.93 (1,400)	99.99 (18,000)	99.99 (470,000)	99.93 (1,400)
	85	99.99 (14,000)	99.83 (580)	99.99 (8,100)	99.99 (83,000)	99.83 (580)
	75	99.99 (9,100)	99.73 (360)	99.98 (5,400)	99.99 (33,000)	99.73 (360)
1:10,000	80	99.99 (45,000)	99.89 (910)	99.99 (26,000)	99.99 (210,000)	99.89 (910)
	70	99.99 (31,000)	99.84 (630)	99.99 (19,000)	99.99 (98,000)	99.84 (630)
	60	99.99 (24,000)	99.79 (480)	99.99 (15,000)	99.99 (57,000)	99.79 (480)
1:31,000	50	99.99 (60,000)	99.85 (690)	99.99 (41,000)	99.99 (120,000)	99.85 (690)
	40	99.99 (51,000)	99.83 (580)	99.99 (37,000)	99.99 (83,000)	99.83 (580)
	30	99.99 (44,000)	99.80 (500)	99.99 (33,000)	99.99 (62,000)	99.80 (500)

[1] Negative predictive value (expressed as a percentage) is the proportion of negative test results associated with a non-cystic fibrosis fetal genotype. The accompanying odds are often referred to as the 'residual risk' and are the odds for having an affected fetus given a negative test result.

N/NT - one partner negative, the other was not tested
P/N - one partner positive, the other was negative
CNP – the couple was not screen positive
N/N – both partners were negative

Prenatal screening models and initial positive rates

The initial aim of prenatal screening may be defined as identifying carrier couples (Table 3-2), but that is often not done in a single step. Three screening models have been employed in pilot trials (Question 33) and will discussed in more detail, later.

- The two-step (or sequential) model first tests the woman's sample. If a mutation is identified, the woman and her partner are contacted and the partner's sample is collected and tested.
- The one-step (or couple) model calls for samples to be collected from both partners at the outset. The woman's sample is usually tested first. When a mutation is identified, the partner's sample can be tested without re-contacting the couple. Unless both partners are carriers, the test is considered negative.
- The expanded one-step (or concurrent) model also requires that samples be collected from both partners, but all samples are tested, and all carriers are notified of their status.

Figure 3-12 graphically displays the sequence of testing for each of these models in a population of 100,000 non-Hispanic Caucasians (the derivation of these numbers can be found in Appendix F). All three models identify the same 118 carrier couples. Overall, 30 of the 40 fetuses are detectable, yielding a clinical sensitivity of about 75 percent. Among the remaining couples in which both partners are not carriers, the number of 'positive' test results varies considerably by screening model chosen. The number of 'false positives' is relatively high in this modeling, as the observed analytic specificity of 0.005 is used. If used routinely, confirmatory testing is likely to considerably reduce this rate.

Clinical performance estimates when the endpoint of screening is considered to be the diagnosis of an affected fetus rather than the identification of a carrier couple From the public health or epidemiologic viewpoint, identifying carrier couples is an intermediate stage of the screening process. Neither of the partners will have health problems due to their carrier status. The final stage of prenatal cystic fibrosis screening (Figure 3-12) is to identify fetuses with two mutations, thereby allowing couples to make decisions about planning for the birth of an affected child or considering the option of pregnancy termination. All three screening models identify the same affected fetuses. The differences are in the numbers of couples who will be made aware of their carrier status and counseled. That rate is 3.9 percent in the two-step model, 0.12 percent for the one-step model, and 7.7 percent for the expanded one-step model. Both false positive and false negative results will occur, but the rates are highly dependent on individual laboratory performance and whether confirmatory testing is performed routinely. A detailed derivation of the data in Figure 3-12 is contained in Appendix F.

Figure 3-12. Prenatal Cystic Fibrosis Clinical Screening Performance in 100,000 non-Hispanic Caucasians According to Three Models

Population Subgroup Tested	Initial Positive Test Results	Partner Tests Positive	Couple Positive	Diagnostic Testing
Clinical sensitivity among 160 non-Hispanic Caucasian carrier couples				
Two-Step (Sequential)				
160 Carrier Couples	→ 138 Women	→ 119 Partners	→ 119 Couples	→ 30 Fetuses Detected
One-Step (Couple)				
160 Carrier Couples	→	→	→ 119 Couples	→ 30 Fetuses Detected
Expanded One-Step (Concurrent)				
160 Carrier Couples	→ 157 One or Both	→	→ 119 Couples	→ 30 Fetuses Detected
Clinical specificity among 99,840 non-Hispanic Caucasian couples that are not both carriers				
Two-Step (Sequential)				
99,840 Not Carrier Couples	→ 3,791 Women	→ 19 Partners	→19 or fewer Couples	→ 0 Fetuses Detected
One-Step (Couple)				
99,840 Not Carrier Couples	→	→	→19 or fewer Couples	→ 0 Fetuses Detected
Expanded One-Step (Concurrent)				
99,840 Not Carrier Couples	→ 7,506 One or Both	→ 35	→35 or fewer Couples	→ 0 Fetuses Detected

Expected prenatal screening performance in Hispanic Caucasian couples: Figure 3-13 shows the numbers of initially positive test results, along with the numbers of carrier couples identified for each of the three screening models applied to a population of 100,000 Hispanic Caucasians. The same assumptions are used here as for Figure 3-12, and the same three screening models are examined. Here, however, the prevalence of cystic fibrosis is set to 1:13,500, and 72 percent of the mutations are assumed to be identifiable by the panel. All three screening models identify the same four affected fetuses. The differences are in the numbers of individuals who will be made aware of their carrier status and counseled. That rate is 1.7 percent in the two-step model, 0.02 percent for the one-step model, and 3.3 percent for the expanded one-step model. Both false positive and false negative results will occur, but the rates are highly dependent on individual laboratory performance and whether confirmatory testing is performed routinely.

Figure 3-13. Prenatal Cystic Fibrosis Screening Performance in Population of 100,000 Hispanic Caucasians According to Three Models

Population Subgroup Tested	Initial Positive Test Results	Partner Tests Positive	Couple Positive	Diagnostic Testing
Clinical sensitivity among 30 Hispanic Caucasian carrier couples				
Two-Step (Sequential)				
30 Carrier Couples	→ 21 Women	→ 15 Partners	→ 15 Couples	→ 4 Fetuses Detected
One-Step (Couple)				
30 Carrier Couples	→	→	→ 15 Couples	→ 4 Fetuses Detected
Expanded One-Step (Concurrent)				
30 Carrier Couples	→ 27 One or Both	→	→ 15 Couples	→ 4 Fetuses Detected
Clinical specificity among 99,970 Hispanic Caucasian couples that are not both carriers				
Two Step (Sequential)				
99,970 Not Carrier Couples	→ 1,686 Women	→ 8 Partners	→ 8 or fewer Couples	→ 0 Fetuses Detected
One-Step (Couple)				
99,970 Not Carrier Couples	→	→	→ 8 or fewer Couples	→ 0 Fetuses Detected
Expanded One-Step (Concurrent)				
99,970 Not Carrier Couples	→ 3,339 One or Both	→ 14	→ 14 or fewer Couples	→ 0 Fetuses Detected

Expected prenatal screening performance in African American couple:s Figure 3-14 shows the numbers of initially positive test results, along with the numbers of carrier couples identified for each of the three screening models. The same assumptions used for Figure 3-12 are used in this example, and the same three screening models are examined. The prevalence of cystic fibrosis, however, is set to 1:15,000, and 65 percent of the mutations are assumed to be identifiable by the panel. All three screening models identify the same three affected fetuses. The differences are in the numbers of individuals who will be made aware of their carrier status and counseled. That rate is 1.5 percent in the two-step model, 0.01 percent for the one-step model, and 3.0 percent for the expanded one-step model. Both false positive and false negative results will occur, but the rates are highly dependent on individual laboratory performance and whether confirmatory testing is performed routinely.

Figure 3-14. Prenatal Cystic Fibrosis Screening Performance in a Population of 100,000 African Americans According to Three Models

Population Subgroup Tested	Initial Positive Test Result	Partner Testing	Couple Results	Diagnostic Testing
Clinical sensitivity among 27 African American carrier couples				
Two-Step (Sequential)				
27 Carrier Couples	→ 17 Women	→ 11 Partners	→ 11 Couples	→ 3 Fetuses Detected
One-Step (Couple)				
27 Carrier Couples	→	→	→ 11 Couples	→ 3 Fetuses Detected
Expanded One-Step (Concurrent)				
27 Carrier Couples	→ 23 One or Both	→	→ 11 Couples	→ 3 Fetuses Detected
Clinical specificity among 99,973 African American couples that are not both carriers				
Two-Step (Sequential)				
99,973 Not Carrier Couples	→ 1,517 Women	→ 7 Partners	→ 7 or fewer Couples	→ 0 Fetuses Detected
One-Step (Couple)				
99,973 Not Carrier Couples	→	→	→ 7 or fewer Couples	→ 0 Fetuses Detected
Expanded One-Step (Concurrent)				
99,973 Not Carrier Couples	→ 2,897 One or Both	→ 12	→12 or fewer Couples	→ 0 Fetuses Detected

Expected prenatal screening performance in Ashkenazi Jewish couples: Figure 3-15 shows the numbers of initially positive test results, along with the numbers of carrier couples identified for each of the three screening models. The same assumptions used for Figure 11 are used for this example, and the same three screening models are examined. The prevalence of cystic fibrosis, however, is set to 1:2,300, and 94 percent of the mutations are assumed to be identifiable by the panel. All three screening models identify the same 37 affected fetuses. The differences are in the numbers of individuals who will be made aware of their carrier status and counseled. That rate is 4.3 percent in the two-step model, 0.15 percent for the one-step model, and 8.4 percent for the expanded one-step model. Both false positive and false negative results will occur, but the rates are highly dependent on individual laboratory performance and whether confirmatory testing is performed routinely.

Figure 3-15. Prenatal Cystic Fibrosis Screening Performance in 100,000 Ashkenazi Jewish Couples According to Three Models

Population Subgroup Tested	Initial Positive Test Result	Partner Testing	Couple Results	Diagnostic Testing
Clinical sensitivity among 174 Ashkenazi Jewish carrier couples				
Two-Step (Sequential)				
174 Carrier Couples	→ 160 Women	→ 147 Partners	→ 147 Couples	→ 37 Fetuses Detected
One-Step (Couple)				
174 Carrier Couples	→	→	→ 147 Couples	→ 37 Fetuses Detected
Expanded One-Step (Concurrent)				
174 Carrier Couples	→ 173 One or Both	→	→ 147 Couples	→ 37 Fetuses Detected
Clinical specificity among 99,826 Ashkenazi Jewish couples that are not both carriers				
Two-Step (Sequential)				
99,826 Not Carrier Couples	→ 4,158 Women	→ 20 Partners	→20 or fewer Couples	→ 0 Fetuses Detected
One-Step (Couple)				
99,826 Not Carrier Couples	→	→	→20 or fewer Couples	→ 0 Fetuses Detected
Expanded One-Step (Concurrent)				
99,826 Not Carrier Couples	→ 8,236 One or Both	→ 39	→39 or fewer Couples	→ 0 Fetuses Detected

Expected prenatal screening performance in Asian American couples: Figure 3-16 shows the numbers of initially positive test results, along with the numbers of carrier couples identified for each of the three screening models. The same assumptions used for Figure 3-12 are used in this analysis, and the same three screening models are examined. The prevalence of cystic fibrosis, however, is set to 1:31,000, and 49 percent of the mutations are assumed to be identifiable by the panel. All three screening models identify the same affected fetus. The differences are in the numbers of individuals who will be made aware of their carrier status and counseled. That rate is 1.0 percent in the two-step model, <0.01 percent for the one-step model, and 2.0 percent for the expanded one-step model. Both false positive and false negative results will occur, but the rates are highly dependent on individual laboratory performance and whether confirmatory testing is performed routinely.

Figure 3-16. Prenatal Cystic Fibrosis Screening Performance According to Chosen Model in 100,000 Asian American Couples

Population Subgroup Tested	Initial Positive Test Result	Partner Testing	Couple Results	Diagnostic Testing
Clinical sensitivity among 13 Asian American carrier couples				
Two-Step (Sequential)				
13 Carrier Couples	→ 6 Women	→ 3 Partners	→ 3 Couples	→ 1 Fetus Detected
One-Step (Couple)				
13 Carrier Couples	→	→	→ 3 Couples	→ 1 Fetus Detected
Expanded One-Step (Concurrent)				
13 Carrier Couples	→ 9 One or Both	→	→ 3 Couples	→ 1 Fetus Detected
Clinical specificity among 99,987 Asian American couples that are not both carriers				
Two-Step (Sequential)				
99,987 Not Carrier Couples	→ 1,036 Women	→ 5 Partners	→ 5 or fewer Couples	→ 0 Fetuses Detected
One-Step (Couple)				
99,987 Not Carrier Couples	→	→	→ 5 or fewer Couples	→ 0 Fetuses Detected
Expanded One-Step (Concurrent)				
99,987 Not Carrier Couples	→ 2,051 One or Both	→ 7	→ 7 or fewer Couples	→ 0 Fetuses Detected

Appendix F. Computation of Screening Performance for the Three Prenatal Models in non-Hispanic Caucasians

Clinical sensitivity among 160 carrier couples

- For the two-step (sequential) model, 138 (160 * 0.88 * 0.979), carrier women are initially detected and 119 (137.6 *.0.88 * 0.979) of the corresponding partner-carriers are also identified. Among the 119 carrier couples, about 30 (119/4) fetuses affected with cystic fibrosis are expected.
- For the one-step (couple) model, only the 119 carrier couples are identified as having a positive test result and the same 30 fetuses are identified.
- For the expanded one-step (concurrent) model, all but the 3 couples with two unidentifiable mutations ($160 * (1-0.88 * 0.979)^2$) will have at least one partner with a positive test result. However, the same 119 carrier couples and 30 affected fetuses will be identified.

Clinical specificity among 99,840 non "carrier couples".

Assuming a carrier frequency of 1/25, 7,680 couples will consist of one true carrier and one true non-carrier partner. In the remaining 92,160 couples, both will be true non-carriers. All analyses assume that the 'false positive rate' is 0.005 (0.5 percent) (e.g., analytic specificity of 99.5%).

- Applying the two-step model to the 7,680 couples yields 3,308 women with true positive tests (7,680 / 2 * 0.88 * 0.979). Among the remaining 4,372 women tested, 22 false positive results will occur (4,379 * 0.005). Among the 3,330 (3,308+22) partners tested, no true positives and an estimated 17 false positive results will occur (3,330 * 0.005). No affected fetuses will be identified among these 17 false positive couples. Applying the two-step model to the 92,160 couples in whom none are carriers will yield initial false positive results among 461 women (92,160 * 0.005). Among their partners, two will have a positive test (461 * 0.005). Overall, 3,791 women will initially be identified as being positive (3,308 true positives and 22 + 461 false positives). Up to 19 false positive couples will be reported; none will have an affected fetus identified.
- Applying the one-step model to the same groups will yield the same 19 false positive couples, but all remaining couples would receive negative test results, as the remaining women with false positive results will have partners with no mutation identified. Among the 19 false positive couples, none will have an affected fetus identified.
- Applying the expanded one-step model to the 7,680 couples yields 6,616 (7,680 * 0.88 * 0.979) couples where one partner is a true positive. Of these, 6,583 (6616 * 0.995) will find one partner to be a true negative, but in 33 (6,616 * 0.005) the other partner will be a false positive and the couple will be incorrectly reported as a positive couple. Among the remaining 1,064 couples, five (1,064 * 0.005) will be incorrectly reported as one positive and one negative. The remaining 1,059 (1064 – 5) couples will be found to be both negative. Applying the expanded one-step model to the 92,160 couples in whom none are carriers will initially yield 461 false positive test results and 2 (461 * 0.005) of these will result in a false positive carrier couples. In the remaining 91,699 partner tests, an additional 458 (91,699*.005) false positive tests will occur yielding a total of 917

(459+458) couples with one positive and one negative test result. The remaining 91,241 couples will receive a correct negative/negative report.

CLINICAL VALIDITY

Question 24: What are the genotype/phenotype relationships?

Summary

- The cystic fibrosis phenotype occurs about 98 percent of the time when any combination of two mutations contained in the recommended 25 mutation core panel are identified in an individual.
- Nearly all of the mutations are associated with pulmonary disease (the major cause of morbidity and mortality), but it is not possible to predict the time of onset and rapidity of progression.
- Pancreatic insufficiency is also present in most affected individuals, but 5 to 15 percent retain some level of pancreatic function. A few of the less common mutations are associated with pancreatic sufficiency.
- Screening will generate more information about genotype/phenotype relationships, especially for less common mutations

Phenotype

Cystic fibrosis is a monogenic autosomal recessive disorder. Genotype, typically defined as the presence of two disease-causing mutations on separate alleles, is, therefore, a primary cause of the development of the clinical phenotype. The underlying cause of cystic fibrosis involves abnormal chloride and sodium ion transport resulting from dysfunction of a cell membrane protein, the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*). Disease-causing mutations in the *CFTR* gene on chromosome 7 result in complete or partial loss of functional *CFTR*, and development of a cystic fibrosis phenotype.

Phenotype is defined by the natural history of the disease, including a specific configuration of signs and symptoms, their severity, and the time of presentation. While there is some variability in clinical presentation, cystic fibrosis is a serious, progressive, multi-system disease (Table 3-29). It is characterized by chronic obstructive pulmonary disease, exocrine pancreatic insufficiency, elevated sweat chloride concentration (>60 mM), and infertility in almost all males due to obstructive azoospermia (congenital bilateral absence of the vas deferens or CBAVD). Age of onset of symptoms varies but is generally early. In a minority of cases, infants are diagnosed in the neonatal period due to meconium ileus (i.e., intestinal obstruction due to inspissated secretions). The median age at diagnosis is 6 months, with about 80 percent of cases diagnosed by 3 years of age (CF Foundation 1999 Data Registry). About 5 to 15 percent of affected individuals retain some level of pancreatic function (pancreatic sufficiency). Between 1 and 2 percent individuals classified as having cystic fibrosis are less severely affected. Some have less severe pulmonary disease, or no evidence of pancreatic dysfunction and borderline or normal sweat chloride levels (Rosenstein *et al.,* 1998; Cutting, 2001). Others are described with late onset of symptoms or unusually mild pulmonary disease.

Gap in Knowledge: Unbiased information about the genotype/phenotype relationship is currently limited.
The estimates contained in Table 3-29 are derived from multiple studies and a general consensus has not yet been achieved. Some studies were small and other may have been subject to biases of ascertainment. For example, the Cystic Fibrosis Foundation has data available to perform a survival analysis for cystic fibrosis individuals stratified by pancreatic status. However, such an analysis has not yet been reported.

Table 3-29. Clinical Characteristics of Cystic Fibrosis

Clinical Characteristic	Proportion of Individuals with Cystic Fibrosis Having that Clinical Characteristic (%)
Severe chronic pulmonary disease	98
Elevated sweat chloride levels	90-98
Male infertility	95
Pancreatic insufficiency	
Total	83-94
Partial	5-15
None	1-2

Other medical conditions associated with cystic fibrosis mutations
Several monosymptomatic disorders have been described that are, or may be, *CFTR*-related, including:

- Congenital bilateral absence of the vas deferens (CBAVD)
- Idiopathic pancreatitis
- Disseminated bronchiectasis
- Allergic bronchopulmonary aspergillosis
- Atypical sinopulmonary disease

The purpose of prenatal screening is to identify couples at high risk of having a fetus with the typical features of cystic fibrosis (Table 3-29). For this reason, these unusual and, for the most part, rare conditions are unlikely to be a major consideration in prenatal cystic fibrosis screening.

Genotype
More than 900 mutations in the cystic fibrosis gene have been reported to the Cystic Fibrosis Genetic Analysis Consortium (http://www.genet.sickkids.on.ca/cftr/). Not all have been found to cause disease, and some are known to be benign polymorphisms. The most common mutation, delF508, accounts for 66 percent of cystic fibrosis chromosomes in a worldwide survey (Tsui and Durrie, 1997). Among the more than 15,000 cystic fibrosis patients in the United States genotyped through 1999, 52 percent are homozygous for delF508, and another 36 percent are compound heterozygotes having delF508 and another mutation (CF Foundation, 1999 Data Registry). The next most common mutations worldwide are G542X, G551D, 621+1G>T, N1303K and W1282X, each accounting for 1 to 2.5 percent of mutations. About 20 additional less common mutations occur at frequencies at or above 0.1 percent. All of the rest are rare, with some reported only in one case or within a single family. Mutation frequencies, and,

consequently, the proportion of mutations detectable using a specific panel, vary by race and ethnicity. For more information about mutation frequencies, see Question 18.

Relationship between genotype and phenotype

Cystic fibrosis mutations can be classified by the molecular mechanisms by which they cause dysfunction (Table 3-30) (Kerem and Kerem, 1996; Rosenstein and Zeitlin, 1998; Mickle and Cutting, 1998; Mickle and Cutting, 2000; Zielensky, 2000). Mutations can result in *CFTR* that is absent, reduced, or abnormally functioning. These are grouped, as follows:

- Class I mutations (e.g., G542X, 621+1G>T, and 711+1G>T) result in total deficiency or unstable/non-functional *CFTR* protein.
- Class II mutations (e.g., delF508, N1303K, and delI507) disrupt normal intracellular processing (e.g., glycosylation), causing instability of *CFTR* protein, or interfering with its movement to the correct cellular location.
- Class III mutations (e.g., G551D) result in a normal amount of *CFTR* protein being produced and positioned at the cell surface, but the protein is non-functional.
- Class IV mutations (e.g., R117H, A455E) result in a normal amount of functional *CFTR* at the cell membrane, but chloride conductance is reduced. These mutations are generally associated with a pancreatic sufficiency.
- Class V mutations (e.g., 3849+10KbC>T)result in reduced levels of normally functional *CFTR* protein at the cell membrane and are also associated with a less severe phenotype.

Table 3-30. Mutation Classification by Mechanism of *CFTR* Dysfunction

***CFTR* Mutations Included in the Recommended Panel of 25**		
Mutation Classes I, II, and III		**Mutation Classes IV and V**
delF508	N1303K	R117 H
G542X	711+1G>T	R334W
621+1G>T	delI507	A455E
G551D	R1162X	R347P
W1282X	R560T	3849+10KbC>T
R553X	1078delT	IVS8-5T
1717-1G>A	I148T	G85E
3659delC	2184delA	2789+5G>A
		3120+1G>T

The phenotypic effects of mutations in the first three classes are generally more severe, resulting in chronic pulmonary disease, pancreatic exocrine dysfunction, elevated sweat chloride levels, and CBAVD. Phenotypic severity does not appear to vary significantly for any combination of two mutations from these classes. The last two classes of mutations produce phenotypic effects that are similar to those found for the first three classes with regard to pulmonary disease, but pancreatic function is more often preserved. This is consistent with clinical observations of strong concordance in pancreatic function in affected sibs. Cystic fibrosis patients with Class IV or V mutations may also develop symptoms at a later age (as adolescents or adults). In a small percentage of individuals with two mutations, the presentation is less typical (Kerem and Kerem, 1996; Rosenstein and Zeitlin, 1998; Mickle and Cutting, 1998; Zielenski, 2000). While these

expectations are generally correct, the associations are far from absolute. In a summary of seven published studies, a proportion of affected individuals with Class I, II or III mutations have delayed onset of pancreatic dysfunction, and a third of affected individuals with one or two of the Class IV or V mutations suffer from pancreatic insufficiency (Murray *et al*., 1999; Cutting, 2001).

The R117H mutation and reflexive testing

The R117H mutation is found in about 0.7 percent of chromosomes in non-Hispanic Caucasian individuals affected with cystic fibrosis (Question 18, Table 3-4). Among unaffected individuals in this population group, therefore, an R117H carrier would be expected once in every 3,860 individuals tested (1:27/0.007). Even fewer carriers would be expected in other populations (Question 18, Tables 3.6, 3-8, 3-10 and 3-12). Early pilot trials did not include this mutation in their screening panels. The first pilot trial to report experience with this mutation found 16 R117H carriers among 2,633 non-Hispanic Caucasians tested (carrier rate of 1:166) (Witt *et al.,* 1996). This was nearly 20 times higher than expected. Clearly, most of these individuals were not carriers of a serious mutation. If the R117H mutation is to be used in prenatal screening, it would be necessary to identify conditions under which the mutation contributes to the classic cystic fibrosis phenotype.

Based on analyses of the CFTR gene in affected and unaffected individuals, it was determined that a gene modifier determined the phenotype associated with the R117H mutation when it was combined with another mutation (e.g., delF508) (Kiesewetter, 1993). The impact of R117H is dependent on the length of the polypyrimidine tract located in intron 8. Three length variants have been identified and designated 5T, 7T and 9T. These are found in about 5 percent, 10 percent and 85 percent of the general population, respectively. This Poly-T variant occurs in a noncoding region of the gene several exons removed from the R117H location (exon 4), but it affects gene expression by influencing splicing efficiency. The phenotypic variation is molecularly based and determined by whether the R117H mutation is located on the same (*cis*) or opposite (*trans*) chromosome 7. In order for this mutation to produce a severe phenotype, it must be 1) associated with the 5T variant on the same chromosome (*in cis),* and 2) the other chromosome must also carry a CF mutation that is capable of producing the phenotype. In the setting of prenatal screening, the ACMG has recommended that Poly-T testing be performed only as a reflex test for carriers shown to be heterozygous for the R117H mutation (Grody *et al*., 2001).

For example, a pregnant woman is found to be a carrier of the R117H mutation. Reflexive testing is performed to determine whether she carries the 5T polymorphism. In 95 percent of women tested, the polymorphism will be either 7T or 9T, and the woman can be informed that the mutation will not be associated with classic cystic fibrosis in the fetus, even if the partner if found to be a carrier. No further testing is required. It is necessary to test the parents of the remaining 5 percent of women, to determine whether the 5T polymorphism is in *cis* or *trans* with the R117H mutation. If it is in *trans*, no further testing is necessary and the woman can be informed that the mutation will not be associated with classic cystic fibrosis in the fetus, even if the partner if found to be a carrier. If, however, the 5T is in *cis*, the testing process continues to the next step; obtaining a sample from the partner. In the event that the woman's parents cannot

be tested, testing of her partner could be undertaken with the knowledge that the partner will not have an identifiable mutation in approximately 29 of 30 such instances.

Published recommendations and some laboratory practices have clouded this relatively straightforward process by including a discussion of infertility, due to congenital absence of the vas deferens (CBAVD) in otherwise healthy men (Anguiano *et al*., 1992; Gervais *et al*., 1993; Kiesewetter *et al*., 1993; Dork *et al*., 1997). Several combinations of the R117H mutation and Poly-T have been associated with CBAVD, and this finding can be helpful when investigating infertility. However, testing for the Poly-T in the absence of the R117H mutation can result in placing the couple, the laboratory and the referring physician in a difficult situation. For example, it may necessitate the discussion of possible CBAVD in male fetuses or the possibility of identifying the male partner as being infertile. In addition, misinterpretation of the penetrance of a 5T finding alone can lead to unnecessary diagnostic testing. In spite of all this, laboratories commonly test for Poly-T variants in samples known to be for prenatal screening and report them even when the R117H mutation is not present, thereby creating difficult and complex counseling situations for their clients.

Gastrointestinal problems

Pancreatic exocrine function has been discussed above. Meconium ileus occurs in 15 to 20 percent of newborns with cystic fibrosis, nearly always in association with pancreatic insufficiency. While this complication nearly always occurs in individuals with pancreatic insufficiency, there is no association with specific mutations. Since most individuals with cystic fibrosis and pancreatic insufficiency do not develop meconium ileus, other genetic and/or environmental factors are likely to be involved. Other less common gastrointestinal problems, such as liver disease, and diabetes, are also not associated with genotype but are associated with pancreatic insufficiency. There is evidence that other genetic and/or environmental factors are involved in these diseases as well.

Respiratory problems

Because lung disease is the primary cause of morbidity and mortality in affected individuals, much attention has been paid to possible associations between pulmonary phenotype and *CFTR* mutations. No significant correlation with genotype, or concordance within sibships, has been demonstrated for pulmonary disease. For most genotypes, including those involving delF508, there is considerable variability in pulmonary phenotype expression. However, the vast majority of individuals with two mutations have serious, progressive lung disease. About 24 percent of children and 64 percent of adults with cystic fibrosis have moderate to severe respiratory compromise, as defined by FEV_1 less than 70 percent of predicted. About 80 percent of cystic fibrosis patients are infected with *Pseudomonas aeruginosa* by 18-24 years of age (CF Foundation, 1999 Data Registry). One mutation, A455E, was associated with milder lung disease and late age of onset in Dutch cystic fibrosis patients (Mickle and Cutting, 1998). Population studies show less severe lung disease in patients with mutations associated with pancreatic sufficiency (Table 3-30, Class IV and V), as compared with other mutations (Zielenski, 2000). Overall, however, genotype is a poor predictor of pulmonary outcome.

Reproductive problems

The male reproductive tract is very sensitive to the effects of *CFTR* mutations. Approximately 95 percent of males with cystic fibrosis are infertile (Welsh *et al*., 1995; Cutting, 2001). Fertility in females is reduced, but a reliable estimate is difficult to determine. In a survey of cystic fibrosis centers in 1980, 129 pregnancies were documented in 100 patients resulting in 86 live births (67 percent); one child was affected with cystic fibrosis (Cohen *et al.,* 1980). In 1994, 135 of female cystic fibrosis patients in the United States between the ages of 15 and 41 (3.7 percent) were pregnant (CF Patient Registry 1994). Of these, 58 (43 percent) had already resulted in a live birth, 14 pregnancies were selectively terminated (10 percent), 11 were spontaneously aborted (8 percent), 2 were lost to follow-up (2 percent) and the remaining pregnancies were still ongoing.

References

Anguiano A, Oates RD, Amos JA, Dean M, Gerrard B, Stewart C, Maher TA, White MTS, Milunsky A. 1992. Congenital bilateral absence of the vas deferens: a primarily genital form of cystic fibrosis. *JAMA* **267**:1794-1797.

Chillon M, Casals T, Mercier B, Bassas L, Lissens W, Silber S, Romey MC, Ruiz-Romera J, Verlingue C, Claustres M. 1995. Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. *N Engl J Med* **332**:1475-1480.

Cohen LF, di Sant'Agnese PA, Frielander J. 1980 Cystic fibrosis and pregnancy: A national survey. *Lancet* 2:842-844.

Cutting GR. Cystic Fibrosis. In *Principles and Practice of Medical Genetics*, Churchill Livingstone, New York, DL Rimoin, JM Connor, RE Pyeritz, Eds., 4th Edition, In press.

Cystic Fibrosis Foundation. Patient Registry 1994 Annual Data Report. 1997. Bethesda, MD.

Cystic Fibrosis Foundation. Patient Registry 1996 Annual Data Report. 1997. Bethesda, MD.

Cystic Fibrosis Foundation. Patient Registry 1998 Annual Data Report. 1999. Bethesda, MD.

Cystic Fibrosis Foundation. Patient Registry 1999 Annual Data Report. 2000. Bethesda, MD.

Dork T, Dworniczak B, Aulehis-Schotz C, Wieczorek D, Bhm I, Mayerova A, Seydewitz H, Nieschlag E, Meschede D, Horst J. 1997. Distinct spectrum of CFTR gene mutations in congenital absence of vas deferens. *Hum Genet* **100**:367-377.

Gervais R, Dumur V, Rigot M-M, Lafite J-J, Roussel P, Claustres M, Demaille J. 1993. High frequency of the R117H cystic fibrosis mutation in patients with congenital absence of the vas deferens. *N Engl J Med* **328**:446-447.

Grody et al. 2001. Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. *Genet Med* **3**:149-154.

Hamosh A, FitzSimmons SC, Macek M, Knowles MR, Rosenstein BJ, Cutting GR. 1998. Comparison of the clinical manifestations of cystic fibrosis in black and white patients *J Peds* **132**:255-259.

Kerem B, Kerem E. 1996. The molecular basis for disease variability in cystic fibrosis. *Eur J Hum Genet* **4**:65-73.

Kiesewetter S, Macek M, Davis C, *et al.*. 1993. A mutation in CFTR produces different phenotypes depending on chromosomal background. *Nat Genet* **5**:274-277.

Mickle JE, Cutting GR. 1998. Clinical implications of cystic fibrosis transmembrane conductance regulator mutations. *Clin Chest Med* **19**:443-459.

Mickle JE, Cutting GR. 2000. Genotype-phenotype relationships in cystic fibrosis. *Med Clin North Am* **84**:597-607.

Murray J, Cuckle H, Taylor G, Littlewood OBE, Hewison J. 1999. Screening for cystic fibrosis. *Health Technol Assess* **3**:1-101.

Rosenstein BJ, Zeitlin PL. 1998. Cystic fibrosis. *Lancet* **351**:277-282.

Rosenstein BJ, Cutting GR. 1998. The diagnosis of cystic fibrosis: A consensus statement. *J Peds* **132**:589-595.

The Cystic Fibrosis Genotype-Phenotype Consortium. 1993. Correlation between genotype and phenotype in patients with cystic fibrosis. *N Eng J Med* **329**:1308-1313.

Tsui L, Durie P. 1997. Genotype and phenotype in cystic fibrosis. *Hosp Prac* 32:115-142.

Welsh MJ, Tsui LC, Boat TF, Beaudet AL. 1995 Cystic Fibrosis in The metabolic and molecular bases of inherited disease (seventh edition, Vol III), McGraw-Hill, New York

Zielenski J. 2000. Genotype and phenotype in cystic fibrosis. *Respiration* **67**:117-133.

CLINICAL VALIDITY

Question 25: What are the genetic, environmental or other modifiers?

Summary:
- Factors other than *CFTR* genotype, particularly other genes and environmental influences, are likely to play a role in the natural history of cystic fibrosis
- No genetic, environmental or other modifiers of phenotype have yet been defined
- In the context of prenatal screening, future knowledge of such modifiers could provide information about prognosis or response to treatment that might influence parental decision-making

The described variability in age of onset, progression of pulmonary disease, and survival in individuals with cystic fibrosis suggests that factors other than *CFTR* genotype, particularly genetic background and environmental influences, are likely to play a role in the natural history of the disease in individual patients (Mickle and Cutting, 1998; Mickle and Cutting, 2000). In particular, little correlation has been demonstrated between specific *CFTR* genotypes and the severity of lung disease, a key determinant for prognosis and survival. For that reason, environmental and other genetic factors may play an important role (Mickle and Cutting, 1998; Mickle and Cutting, 2000). Lungs are directly exposed to a variety of environmental factors, including pollutants (e.g., cigarette smoke) and pathogens. Genetically determined factors could influence key events in progression of cystic fibrosis lung disease. Susceptibility to bacterial infections, for example, could be influenced by genes or regions associated with immunity (e.g., MBL, TNFα) and inflammation (e.g., HLA region). These genetic components, as well as other candidate modifier genes implicated in mouse models and human studies, have been targeted for study (Zielenski, 2000). Genetic factors that influence response to therapeutic intervention are also being explored.

In the context of prenatal screening for cystic fibrosis, future knowledge of important modifiers that could significantly affect prognosis or response to treatment might influence parental decision-making. For example, if a specific gene were identified that influenced an affected individual's response to a new effective treatment, prenatal testing for that gene might provide additional information about prognosis.

References

Cutting GR. Cystic Fibrosis. In *Principles and Practice of Medical Genetics*, Churchill Livingstone, New York, DL Rimoin, JM Connor, RE Pyeritz, Eds., 4th Edition, In press.

Mickle JE, Cutting GR. 1998. Clinical implications of cystic fibrosis transmembrane conductance regulator mutations. *Clin Chest Med* **19**:443-459.

Mickle JE, Cutting GR. 2000. Genotype-phenotype relationships in cystic fibrosis. *Med Clin North Am* **84**:597-607.

Murray J, Cuckle H, Taylor G, Littlewood OBE, Hewison J. 1999. Screening for cystic fibrosis. *Health Technol Assess* **3**:1-101.