DISORDER/SETTING

Question 1: What is the specific clinical disorder to be studied?
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DISORDER/SETTING

Question 1: What is the specific clinical disorder to be studied?

The specific clinical disorder is cystic fibrosis. Nearly all individuals with cystic fibrosis have some degree of progressive lung disease with abnormal chest x-rays, abnormal pulmonary function tests, and abnormal sputum. The natural history and variability of presentation is described in more detail in later sections (Question 26). A core panel of 25 mutations (Question 4, Table 1-1) is recommended to be used for screening by the American College of Medical Genetics and the American College of Obstetricians and Gynecologists. All combinations of those mutations can be associated with severe disease. One of the mutations (R117H), however, often is not usually associated with this disorder, and additional testing is necessary. Another mutation (I148T) also seems to be of low penetrance, but it is not yet known how to properly interpret this mutation. More information about the genotype/phenotype relationship can be found in later sections (Question 20 and 24).

Social context of cystic fibrosis carrier testing

Evaluation of prenatal screening for cystic fibrosis must take into account the social context in which testing is done. The carrier state itself has no medical implications and such testing is done solely to assist in reproductive decision-making in a variety of ways. Men and women who know themselves to be carriers before marriage could reduce their risk of having children with cystic fibrosis by deliberating choosing marriage partners who are not known to be carriers of a mutation. When both spouses in a marriage are known to be carriers, the couple could choose not to have children, adopt, use donor sperm (from a donor whose cystic fibrosis carrier testing is negative), or consider pre-implantation genetic testing.

In practice, however, most individuals show limited interest in cystic fibrosis carrier testing prior to a confirmed pregnancy. Current protocols promote cystic fibrosis carrier testing in the United States during pregnancy. In this clinical setting, the primary purpose is to determine the likelihood that the fetus is affected with cystic fibrosis. This context has several ethical, legal and social implications. First, careful attention must be given to the manner in which carrier testing is offered as part of prenatal care. It is important for couples considering prenatal screening to understand that the purpose of the test is to determine the likelihood that fetus is affected. If both parents are found to be carriers, they will be offered prenatal diagnosis. Prenatal diagnosis provides parents the opportunity to consider termination of the pregnancy if the fetus is found to have cystic fibrosis. Without careful patient education about these issues, the offer of screening could appear to be similar to other tests routinely offered during pregnancy (e.g., blood glucose, blood pressure or pH) and thus could be misconstrued as a test intended to help ensure a healthy pregnancy outcome. Prenatal screening for cystic fibrosis is optional in a way that most other routine prenatal tests are not. Tests to determine Rh status, for example, would be highly recommended, to provide the obstetrician with the opportunity to take action if an Rh incompatibility is found. By contrast, prenatal screening for cystic fibrosis and any resulting prenatal diagnosis procedures, are optional interventions, to be pursued only if the couple is informed and interested.

Couples may differ in their interest in prenatal testing, and in the value they place on prenatal diagnostic information. From the couple's perspective, a prenatal diagnosis of cystic fibrosis could have two potential values. For some, the information allows them to prepare for the birth of an affected child. For others, the prenatal diagnosis of cystic fibrosis creates the opportunity to terminate the pregnancy, in order to avoid the birth of an affected child. Because prenatal diagnosis does not currently lead to any medical treatment (i.e., there are no *in utero* treatments to prevent or ameliorate the clinical complications) couples need to be fully informed about the potential outcomes of testing before initiating the testing process.

Prenatal screening for cystic fibrosis and subsequent prenatal diagnosis fall into a subset of medical tests that are performed for personal or social purposes. Although the interventions associated with a screen positive couple (prenatal diagnosis; termination of an affected pregnancy) are considered medical actions, the rationale for doing them is based in personal (family) values, not in medical judgment. Prenatal screening for cystic fibrosis results in a measurable outcome change only if a pregnancy is terminated to avoid the birth of an affected child. In our society, the dominant ethical perspective related to this decision is that reproductive decisions are private matters determined by the autonomous choices of parents. This moral framework dictates that prenatal screening for cystic fibrosis be offered, but not recommended. Parental decisions regarding whether or not to pursue carrier testing, prenatal diagnosis, and pregnancy determination must be respected. The emphasis of traditional genetic counseling on non-directive modes of counseling reflects this perspective.

Studies of genetic counseling, and of medical decision-making in general, point to difficulties in implementing the goal of non-directive counseling particularly in a time limited setting. Empiric observations in genetic counseling settings, for example, suggest that genetic counselors may frequently make directive statements in the course of counseling and/or patients may interpret offering a test with recommending the test. Non-directiveness may be more difficult to maintain in a routine obstetric practice setting, where prenatal screening for cystic fibrosis is now recommended to be offered to all pregnant women, than in a genetics clinic. This is because the time for counseling is likely to be more constrained and because the test is offered in the context of many other prenatal tests. Thus, careful attention needs to be taken to maintaining the ethical standard of *informing* couples of the opportunity for carrier testing, rather than *recommending* a particular course of action.

Another ethical concern is financial and medical access to pregnancy termination. Health plans differ in their coverage of mid-trimester pregnancy terminations, and the availability of facilities providing this procedure varies significantly in different locations. For some couples at risk to have an affected child, this reality may limit their choices and they should be made aware of this possibility prior to consenting to the first stages of the testing process.

Individuals found to be carriers will be encouraged to share this information with their extended family. The reactions of family members can be quite variable and unpredictable. In the case where both members of a couple are carriers and they are faced with decisions about prenatal testing and termination, caution should be advised in when and how to share their test results with family members and their support system.

The types of decisions couples may have to make during the process of prenatal screening for cystic fibrosis can be quite complex. Information should be provided in multiple formats and on multiple occasions to increase the likelihood that couples will have an opportunity to make an informed decision.

Gaps in Knowledge

1. Have individuals or couples found to be cystic fibrosis carriers experienced insurance discrimination or employment discrimination? Have they suffered from "job lock" because of inability to get private health insurance if they leave their current job?
2. There have been reports of insurers wanting to deny pediatric coverage for cystic fibrosis for a child diagnosed by prenatal testing where the parents elected not to terminate. Have families been pressured into abortion by insurers because they did not know they could resist pressure to abort? Have insurers denied coverage for prenatal cystic fibrosis screening unless couples agreed to abort an affected pregnancy?
3. In some sectors of the Jewish community, there were reports that individuals labeled as carriers might be seen as a less suitable candidate in arranged marriages. How common is this? Have there been successful efforts to reduce or eliminate this perception?
4. Individuals identified as cystic fibrosis carriers are often encouraged to share their carrier status with extended family members. How often does this actually happen? Not all family members want to know this information. Are there documented reports of families ostracizing carriers who share their status?

DISORDER/SETTING

Question 2: What are the clinical findings defining this disorder?

Cystic fibrosis is associated with major health problems and reduced life expectancy. It is one of the most common recessively inherited serious single gene disorders in non-Hispanic Caucasians. In that population group, about 1 in 25 (4%) are carriers, and the birth prevalence of cystic fibrosis is about 1 in 2500. The exocrine glands of affected individuals produce abnormally thick secretions of mucus. This abnormal mucus leads to a wide variety of progressive respiratory and gastrointestinal problems, and impaired fertility in males. Cystic fibrosis does not affect the sexual functioning of either men or women. The long-term prognosis has improved substantially during the past quarter century due to more effective treatment, coupled with the availability of centralized cystic fibrosis management units. Over 90% of affected infants now survive beyond 1 year, and the current median age of death has increased to about 30 years (Cunningham and Taussig, 1999). There is considerable variability in the rate of disease progression and extent of pancreatic involvement, but nearly all homozygous individuals develop serious clinical manifestations at some point. The median predicted survival for individuals with cystic fibrosis is 32.2 years (CF Foundation Registry Annual Report, 2000). Treatment is presently directed at slowing the progress of the disease. Gene therapy offers promise for the future, but has not yet been successfully accomplished. For a more in-depth description of the cystic fibrosis phenotype, see Question 26.

DISORDER/SETTING

Question 3: What is the clinical setting in which the test is to be performed?

In the prenatal setting, pregnant women and their partners are the target group to be offered prenatal screening for cystic fibrosis. This is accomplished by identifying couples in whom both partners are carriers of a cystic fibrosis mutation (carrier testing). Such screening is offered in the physician office or clinic, as part of prenatal care. Information about cystic fibrosis screening is provided as early in pregnancy as possible, preferably at, or before, the first visit (e.g., in a brochure mailed before the initial prenatal care appointment). For those who opt to be tested, samples are obtained and processed promptly. Although targeted in the first trimester, testing can practically be offered as late as 20 weeks. After that time, the options available to the parents may be limited to preparing for management of an affected child. Carrier couples who are identified are offered genetic counseling and diagnostic testing of the fetus.

DISORDER/SETTING

Question 4: What DNA test(s) are associated with this disorder?

Background

The cystic fibrosis gene is located on the long arm of chromosome 7. It spans some 230 kb of genomic DNA and contains 27 exons. The gene product, the cystic fibrosis transmembrane conductance regulator (*CFTR*), is expressed in exocrine glands of those tissues primarily involved in the disease process, such as pancreas, nasal polyps, lung, colon, sweat glands, placenta, liver, and parotid gland. It is a protein with a calculated molecular mass of 170,000, showing strong homology with a class of ATP-dependent membrane transport proteins (Kerem *et al.*, 1989; Riordan *et al.*, 1989; Rommens *et al.*, 1989). Tracking the cystic fibrosis gene through protein assays is impractical, because *CFTR* is not expressed in accessible tissues such as red or white blood cells.

To date, over 900 different disease-causing mutations have been found in this gene, and these vary in frequency according to geographical and ethnic background (Brock, 2000). Many mutations are extremely rare and have been found in one or a handful of patients. The predominant mutant allele at the CF locus is a three base-pair deletion in exon 10, which removes a phenylalanine residue at position 508 of the 1480 amino acid sequence of *CFTR*. This allele, known as delF508 (F is the single letter code for phenylalanine), makes up 50% to 90% of mutations in Caucasian populations (The Cystic Fibrosis Genetic Analysis Consortium, 1990; Schwarz *et al.*, 1995). A different mutation is the main cause of cystic fibrosis in Ashkenazi Jews. Half of Ashkenazi Jewish carriers of cystic fibrosis have the W1282X mutation (rarely found in non-Jewish carriers), whereas less than one-third have the delF508 mutation. In other populations, no single mutation accounts for a dominant proportion.

Laboratory Tests

It is possible to detect both normal and mutant cystic fibrosis alleles in any tissue sample which contains nucleated cells; in practice, this means any tissue with the exception of mature red blood cells. Screening can be successfully done using either white blood cells (Mennie *et al.*, 1992; Schwartz *et al.*, 1993; Jung *et al.*, 1994; Brambati *et al.*, 1996; Brock 1996; Cuckle *et al.*, 1996; Loader *et al.*, 1996; Witt *et al.*, 1996; Eng *et al.*, 1997) or buccal samples (Mennie *et al.*, 1992; Harris *et al.*, 1993; Miedzybrodzka *et al.*, 1995; Brambati *et al.*, 1996; Brock 1996; Cuckle *et al.*, 1996; Harris *et al.*, 1996; Hartley *et al.*, 1997; Grody *et al.*, 1997). Blood samples (obtained by venipuncture) serve as a highly reliable source for DNA and can be readily obtained in many health care settings. The method of collecting buccal cells by brush, "scoop" or mouthwash is inexpensive and is well suited to collecting samples in primary care settings or at home (Harris *et al.*, 1993; Livingstone 1994; Miedzybrodzka *et al.*, 1995; Wald *et al.*, 1995; Brock 1996; Doherty *et al.*, 1996; Harris *et al.*, 1996; Hartley *et al.*, 1997; Bradley *et al.*, 1998), which may have advantages for aspects of the informed consent process and for the ability to obtain simultaneous samples from both parents. Buccal samples are stable when shipped at ambient temperature, and testing has been successfully performed on buccal lysates stored frozen for 3-4 years (Haddow *et al.*, 1999). Buccal sample failure rates are generally 1% or less; results can nearly always be obtained from blood samples. When repeat samples are needed, they can nearly always be ob-

tained. Blood spots on filter paper cards have also been used as a sampling method (Clayton *et al.*, 1995), but details of performance have not been reported.

Of the wide variety of testing methodologies available, most screening trials have chosen forward dot-blot, reverse dot-blot, or amplification refractory mutation system (ARMS™) technologies. All appear reliable, require only a small capital investment, support a reasonable throughput of samples, can test either purified DNA or buccal lysates, and require only a moderate level of technical skill. Current estimates of unit reagent costs are about $30 to $50 per test. In the United States, however, no kits have been approved by the FDA for cystic fibrosis testing, and none is known to be under review. Laboratories offering such testing will likely come under regulations for 'home brew' or, possibly, analyte specific reagents. More automated methodologies are being developed; these may improve capacity and throughput and reduce some costs (e.g., less technologist time), but may require significant capital investment. Examples include: ARMS™, fluorescent assay (Zeneca Diagnostics, Cheshire, England), automation of reverse dot-blot strips (Linear Array CF-31 from Roche Molecular Systems, Alameda, CA), INNO-LiPA CFTR29 (Innogenetics, Alpharetta, GA), and PCR-OLA, a polymerase chain reaction followed by an oligonucleotide ligation assay and a sequence coded separation (PE Biosystems, Foster City, CA). The American College of Obstetricians and Gynecologists/American College of Medical Genetics (ACOG/ACMG) Task Force has recommended that prenatal screening utilize all DNA mutations which occur in 0.1% or more of the affected pan-ethnic American population (www.faseb.org/genetics/acmg/pol-32.htm). The 25 mutations in that category are listed in Table 1-1. A discussion about mutational panels that contain fewer (or more) than the 25 suggested mutations is provided later, as part of the review of mutation frequencies in specific racial/ethnic groups (Question 18).

Table 1-1. Recommended Core Mutation Panel for General Population Cystic Fibrosis Prenatal Screening in the United States*

delF508	delI507	G542X	G551D	W1282X
N1303K	R553X	G21+1G>T	R117H	1717-1G>A
A455E	R560T	R1162X	G85E	R334W
R347P	711+1G>T	1898+1G>A	2184delA	1078delT
3849+10kbC>T	2789+5G>A	3659delC	I148T	3120+1G>A

Reflex Tests:
I506V*, I507V*, delF508C* 5T/7T/9T**

*Benign Variants. This test distinguishes between a cystic fibrosis mutation and these benign variants. I506V, I507V, and delF508C are performed only as reflex tests for unexpected homozygosity for delF508 and/or delI507.
**5T in cis can modify R117H phenotype or alone can contribute to congenital bilateral absence of vas deferens (CBAVD); 5T analysis is performed only as a reflex test for R117H positives.

*Table taken directly from Grody *et al*., 2001.

DISORDER/SETTING

Question 5: Are preliminary screening questions employed?

The pros and cons of using an inquiry about racial/ethnic heritage as an initial screening test were discussed extensively at a working conference in 1998 that dealt with issues in implementing prenatal screening for cystic fibrosis (Haddow *et al.*, 1999a; Haddow *et al.*, 1999b), as well as in a follow-up workshop to the NIH Consensus Development Conference on Cystic Fibrosis (Mennuti and Press, 1998). Both the population prevalence of cystic fibrosis and the frequency of identifiable mutations vary greatly, depending upon race and ethnicity (NIH Consensus Development Conference Statement. Genetic Testing for Cystic Fibrosis). Ashkenazi Jewish and other non-Hispanic Caucasian populations have the highest prevalences, followed by Hispanic, African American and Asian American populations. Cystic fibrosis testing has the highest detection rate in Ashkenazi Jews, followed closely by other non-Hispanic Caucasians. Lower rates are found for African Americans, Hispanics and Asian Americans.

Given this wide variation in cystic fibrosis prevalence (Question 21) and mutation mix between populations (Question 18), it is most effective to offer prenatal cystic fibrosis screening to couples who are Caucasian and of European or Ashkenazi Jewish descent, and who are planning a pregnancy or who are seeking prenatal care during the first or early second trimester. Cystic fibrosis screening could also be made available to racial and ethnic groups that are at lower risk. These couples could be provided with materials that make clear their risk for having a child with cystic fibrosis and the sensitivity and specificity of the available tests. The limitations of prenatal screening in their racial/ethnic group should be addressed and, if they understand this information and request testing, it should be provided.

One way to understand the impact of population prevalence and percentage of identifiable mutations is to determine the number of couples who need to be screened per case detected. It would be necessary to screen 3,500 non-Hispanic Caucasian couples to identify one case of cystic fibrosis [population prevalence divided by the product of the proportion of identifiable mutations in the two partners, or $[2{,}500 / (0.85)^2]$ (Holmes *et al.*, 1998). For Ashkenazi Jewish, Hispanic, African American, and Asian American populations, one case would be identified for every 2,800, 24,000, 27,000 and 333,000 couples screened, respectively (Haddow *et al.*, 1999). Answers to a question about racial/ethnic heritage in a screening setting have proven useful in distinguishing between these various levels of risk, even though it is sometimes difficult to make these assignments (Question 23).

In order to implement this approach, two strategies might be considered:

- Prenatal screening for cystic fibrosis might include a preliminary screening question about racial/ethnic heritage, as is now the accepted practice for some other genetic disorders, such as Tay-Sachs disease. Only non-Hispanic Caucasians and Ashkenazi Jewish individuals would be offered screening. Such a strategy might be controversial.
- Educational materials would make clear to women and couples the prevalence and screening performance in various racial/ethnic groups. Among those low risk groups that have poor screening performance, it is likely that many will decline (Loder *et al.*, 1996).

DISORDER/SETTING

Question 6: Is it a stand-alone screening test or is it one of a series of screening tests?

The DNA testing that is used for screening couples prenatally to detect cystic fibrosis in the fetus is a "stand-alone" laboratory test. It may be proceeded in some programs by a screening question concerning race/ethnicity that is used to determine which couples would be offered screening. Other programs may provide race- and ethnic-specific test performance estimates and leave the decision about test acceptance up to the couple.

DISORDER/SETTING

Question 7: If it is part of a series of screening tests, are all tests performed in all instances (parallel) or are only some tests performed on the basis of other results (series)?

If a screening question about race/ethnicity is used initially then DNA testing will be performed only on those identified as suitable candidates. In this instance, the DNA testing will be performed on the basis of the preliminary screening test and, therefore, the tests are performed in series.

References

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.

Brock DJH. 2000. Cystic fibrosis. *In:* Antenatal and Neonatal Screening. Second Edition. Wald N & Leck I (eds.). Oxford University Press: London, pp. 325-349.

Clayton EW, Hannig VL, Pfotenhauer JP, Parker RA, Campbell PW, Phillips JA. 1995. Teaching about cystic fibrosis carrier screening by using written and video information. *Am J Hum Genet* **57**:171-281.

Couper RTL, Corey M, Moore DJ, Fisher J, Forstner GG, Durie PR. 1992. Decline of exocrine pancreatic function in cystic fibrosis patients with pancreatic sufficiency. *Pediatr Res* **32**:170-182.

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.

Cunningham JC, Taussig LM. 1999. *In:* An Introduction to Cystic Fibrosis for Patients and Families. Cystic Fibrosis Foundation, p. 99.

Cystic Fibrosis Foundation, Patient Registry 2000 Annual Report, Bethesda, Maryland, September 2001.

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, 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**:149-54.

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

Haddow JE, Bradley LA, Palomaki GE, Doherty RA, Bernhardt BA, Brock DJH, *et al*. 1999b. Issues in implementing prenatal screening for cystic fibrosis: Results of a working conference. *J Med Screen* **6:**60-66.

Haddow JE, Palomaki GE, Bradley LA, Doherty RA. 1998. Screening for cystic fibrosis. *JAMA* **279**:1068.

Harris H, Scotcher D, Hartley N, Wallace A, Craufurd D, Harris R. 1993. Cystic fibrosis carrier testing in early pregnancy by general practitioners. *BMJ* **306**:1580-1583.

Harris H, Scotcher D, Hartley N, Wallace A, Craufurd D, Harris R. 1996. Pilot study of the acceptability of cystic fibrosis carrier testing during routine antenatal consultations in general practice. *Br J Gen Pract* **46**:225-227.

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

Holmes LB, Pyeritz RE (for the Clinical Practice Committee of the American College of Medical Genetics). 1998. Screening for cystic fibrosis. *JAMA* **279**:1068-1069.

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.

Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC. 1989. Identification of the cystic fibrosis gene: genetic analysis. *Science* **245**:1073-1080.

Livingstone J, Axton RA, Gilfillan A, Mennie M, Compton M, Liston WA, Calder AA, Gordon AJ, Brock DJH. 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 Jr., Rowley PT. 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, Whyte DA, Brock DJ. 1992. Prenatal screening for cystic fibrosis. *Lancet* **340**:214-216.

Mennuti M, Thomson E, Press N. 1999. Screening for cystic fibrosis carrier state. *Obstet Gynecol* **93**:456-461.

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

NCCLS Molecular diagnostic methods for genetic disease; Approved guideline (MM1-A) 2000;17:2,12. Wayne, Pennsylvania.

New York State Department of Health, Clinical Laboratory Evaluation Program, Laboratory Standards Rev 9/00 (www.wadsworth.org/labcert/download.htm).

NIH Consensus Development Conference Statement. Genetic testing for cystic fibrosis. Available at http://odp.od.nih.gov/consensus/statements/cdc/106/106_stmt.html. Accessed June 17, 2002.

Notice of Intent; Genetic testing under the clinical laboratory improvement amendments. 2000. *Federal Register* **65**:24928-24934.

Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, *et al.* 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* **245**:1066-1073.

Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy K, Hidaka N, *et al.* 1989. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* **245**:1059-1065.

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.

Schwarz MJ, Malone GM, Haworth A, Cheadle JP, Meredith AL, Gardner A, Sawyer IH, Connarty M, Dennis N, Seller A, *et al.* 1995. Cystic fibrosis mutation analysis: report from 22 UK regional genetics laboratories. *Hum Mutat* **6**:326-333.

Tanner MS. 1992. Liver and biliary problems in cystic fibrosis. *J R Soc Med* **85**:20-24.

The Cystic Fibrosis Genetic Analysis Consortium. 1990. Worldwide survey of the deltaF508 mutation. *Am J Hum Genet* **47:**354-359.

Wald NJ, George L, Wald N, MacKenzie 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, 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.