

Cystic Fibrosis (*CFTR* gene) Mutation Testing

The UNC Hospitals Molecular Genetics Laboratory performs a polymerase chain reaction oligonucleotide ligation assay (PCR/OLA) on the *CFTR* gene to detect 32 common mutations associated with cystic fibrosis. Additional mutation analysis is done under selected circumstances as described below.

Disease Pathogenesis: Cystic fibrosis (CF) is an autosomal recessive disorder affecting multiple organ systems. The leading cause of morbidity and mortality is progressive decline in pulmonary function resulting from airway damage caused by thickened secretions complicated by chronic microbial infection. In addition, ~85% of CF patients develop insufficiency of the exocrine pancreas that necessitates lifelong administration of dietary enzyme supplements. Other complications include meconium ileus in ~15% of CF patients, diabetes mellitus in 15%, and severe liver disease in ~5%. In addition, 99% of CF males are infertile because of congenital bilateral absence of the vas deferens (CBAVD). Most CF patients have elevated sweat chloride values which, along with genetic testing, support a clinical diagnosis. CF is common among Caucasians of Northern European descent, with about 1/2500 affected and a carrier rate of about 1/25. Other ethnic and racial groups are less commonly affected. For example, the prevalence of CF among African-Americans is approximately 1/17,000, which corresponds to a carrier rate of 1/65. On a molecular level, a defective *CFTR* protein leads to inadequate transport of chloride ions between the intra- and extracellular environments of epithelial cells in affected organs. In the sinopulmonary tract, this leads to dehydration of the thin mucous layer lining airways, creating an environment promoting bacterial colonization. In pancreatic ducts, the same defect leads to thickened secretions blocking the duct and preventing transport of pancreatic enzymes into the digestive tract. The biliary tree, vas deferens, and sweat ducts are likewise compromised.

Genotype: CF is characterized by substantial allelic heterogeneity, with more than 1000 different mutations reported within the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Approximately 50% of Caucasian CF patients are homozygous for the deltaF508 mutation which results in complete loss of *CFTR* function and classic, severe manifestations of the disease. About 40% of CF patients have deltaF508 on one chromosome and another less common mutation on the other chromosome. The remaining ~10% have two rare mutations. Among African-American CF patients, a splicing mutation (3120+1 G>A) accounts for about 12% of mutated alleles. Some of the uncommon mutations are associated with residual chloride transport capacity and a milder clinical course. However, since there is substantial clinical variability among CF patients with identical genotypes, it is not possible to predict individual patient outcome on the basis of *CFTR* genotype alone.

Indications for Molecular Testing: CF mutation testing is performed for the purpose of diagnosis of CF or to determine carrier status. Indications include clinical suspicion either of CF (mild, severe, or atypical forms), CBAVD, or idiopathic chronic pancreatitis. The American College of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics (ACMG) recommend offering testing for 23 common *CFTR*

mutations to all women currently planning a pregnancy or seeking prenatal care. This helps identify carriers and thereby informs their reproductive choices. Each of the 23 mutations in the ACOG/ACMG panel has an allele frequency of 0.1% or greater in the general, pan-ethnic U.S. population. The panel of tests offered at UNC Hospitals includes the recommended 23 mutation panel (in boldface type) plus an additional nine mutations as listed in *Table 1*:

Table 1:

deltaF508	N1303K	A455E	1078delT	S549N
deltaI507	R553X	R560T	711+1G>T	S549R
G542X	621+1G>T	R1162X	1898+1G>A	V520F
G551D	R117H	G85E	2184delA	3876 delA
W1282X	1717-1G>A	R334W	R347P	R347H
3849+10kbC>T	2789+5G>A	3659delC	2183AA>G	394delTT
3120+1G>A	3905insT			

Molecular Testing: The preferred sample type is ACD or EDTA anticoagulated blood (pale yellow top or lavender top, 3ml), which may be refrigerated up to 48 hours before analysis. A mutation panel is tested by PCR followed by oligonucleotide ligation assay using commercial analyte specific reagents (ASRs) available through Abbott diagnostics that were validated in our laboratory. In diagnostic (as opposed to screening) tests, three polymorphisms that have the potential to confound mutation analysis are also evaluated along with analysis of the intron 8 polypyrimidine tract polymorphism (5T, 7T and 9T). Intron 8 analysis is also done as a reflex test when an R117H mutation is detected because 5T in *cis* with R117H is a disease-related allele. Individuals of Ashkenazi Jewish descent are tested for D1152H in addition to the routine panel of mutations. Testing for D1152H is performed by the Third Wave InPlex™ CF Assay. Results are reported as either consistent with a diagnosis of CF (two mutations found); at least carrier status (one mutation identified); or no detectable mutation (which reduces the probability of CF or carrier status). We recommend that screening (carrier testing) of the partner of a carrier be sent to an outside laboratory where more extensive mutation testing is possible. When screening is simultaneously requested on both partners, testing is done in-house.

Genetic Counseling: Genetic Counselors and Geneticists are available to discuss testing options and the clinical implications of test results. To make a patient appointment, call 966-2229. Counselors can advise individuals of different ethnic or racial backgrounds of their *a priori* risk for CF carrier status. The unique spectrum of mutations within each sub-population corresponds to a different test detection rate for the 23-mutation panel in each group. Approximate carrier rates and risks are summarized in *Table 2* below.

Table 2:

<u>Sub-population</u>	<u>Carrier Detection Rate</u>	<u>Risk Before Test</u>	<u>Risk After Negative Test</u>
Ashkenazi Jewish	97%	1/29	1/930
European Caucasian	80%	1/29	1/140
African American	69%	1/65	1/207
Hispanic American	57%	1/46	1/105
Asian	unknown	1/90	unknown

Pre- and Post-analytic aspects of screening using the ACOG/ACMG panel:

Typically couples are informed of the availability of CF testing through the woman's obstetrician, gynecologist, or primary care physician. If testing is desired, the woman's blood is drawn and tested with the CF mutation panel. There are several possible outcomes and recommended courses of action:

- a. Screening test is negative for *CFTR* mutations. The result is reported as no detectable mutation, and typically no further testing is performed.
- b. Test is positive for two *CFTR* mutations. The patient is at least a carrier of CF and may also have clinical signs of disease. The patient should be counseled regarding the clinical implications of this genotype as well as its impact on reproductive risks. See (c) below regarding testing of the partner.
- c. Test is positive for one *CFTR* mutation. The patient is a carrier of CF. The patient should be counseled that the risk of having a child with CF is significantly higher than the population-based risk calculated prior to testing. Mutation testing of the partner is now indicated. Blood is collected from the partner and sent to the UNC Hospitals Lab along with a requisition specifying that it is from the partner of a patient known to carry a *CFTR* gene mutation. Our policy is to send the sample to an outside laboratory for extended mutation testing. A negative result with this extended panel reduces residual carrier risk in the partner to a greater degree than what is achieved by the routine mutation panel. Alternatively, testing at UNC for the routine panel of mutations may be requested with a turn-around time of 1 week, compared to 3 weeks for send-out testing. This choice is especially relevant for a couple with an ongoing pregnancy approaching 20 weeks gestation.

References:

1. Online Mendelian Inheritance in Man:

<http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispim?219700#sectRF>

2. Gene Reviews: <http://genetests.org>

3. American College of Medical Genetics: <http://www.acmg.net/>

4. Grody WW et al: Laboratory Standards and Guidelines for Population-based Cystic Fibrosis Carrier Screening. *Genetics in Medicine* 3: 149-154, 2001.

<http://www.faseb.org/genetics/acmg/pol-32.htm>

Questions?

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