Cystic Fibrosis (*CFTR* gene) Mutation Testing

The UNC Hospitals Molecular Genetics Laboratory performs genotyping of the *CFTR* gene to detect 60 common mutations associated with cystic fibrosis.

Disease Pathogenesis: Cystic fibrosis (CF) is an autosomal recessive heritable disorder affecting multiple organ systems. While carriers are unaffected, among homozygotes the leading cause of morbidity and mortality is progressive decline in pulmonary function resulting from thickened secretions in damaged airways, complicated by chronic microbial infection. ~85% of CF patients develop pancreatic insufficiency necessitating lifelong dietary enzyme supplements. Other complications include meconium ileus in ~15%, diabetes mellitus in 15%, and severe liver disease in ~5%. 99% of males are infertile due to congenital bilateral absence of the vas deferens (CBAVD).

Mechanistically, mutant CFTR protein leads to inadequate transport of chloride ions in epithelial cells of affected organs. In the sinopulmonary tract, this leads to dehydration of the mucous layer lining airways, creating an environment promoting bacterial colonization. In pancreatic ducts, thickened secretions can block the duct and diminish transport of pancreatic enzymes into the digestive tract. The biliary tree, vas deferens, and sweat ducts are likewise compromised. Most CF patients have elevated sweat chloride values supporting a clinical diagnosis.

Genotype and phenotype: CF is characterized by substantial allelic heterogeneity, with more than 1000 different pathogenic mutations reported in the ***cystic fibrosis transmembrane conductance regulator (CFTR)*** gene. CF is common among White. Whites 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. Approximately 50% of White CF patients are homozygous for the deltaF508 mutation which results in complete loss of CFTR function and classic, severe manifestations. Among African-American CF patients, a splice mutation (3120+1 G>A) accounts for about 12% of mutated alleles. Less common 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 *CFTR* Molecular Testing:

1. **To help diagnose cystic fibrosis** including mild or atypical forms; CBAVD; or hereditary pancreatitis
2. **To determine carrier status:** The American College of Obstetricians and Gynecologists and the American College of Medical Genetics and Genomics recommend offering tests for 23 common *CFTR* mutations to determine carrier status in women currently planning a pregnancy or seeking prenatal care. Each of the 23 mutations has an allele frequency of 0.1% or greater in the general, pan-ethnic U.S. population. The panel of mutations tested at UNC Hospitals includes these 23 mutations (boldfaced), plus 37 more mutations to improve detection in non-white ethnicities (Table 2). Relatives may qualify for screening of a known familal mutation.

Molecular Testing: A Luminex bead hybridization assay is performed on blood. 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. The *CFTR* gene is analyzed for 60 mutations by polymerase chain reaction followed by allele specific primer extension and bead hybridization (Luminex xTAG® Cystic Fibrosis 60 kit v2). This assay has been cleared by the US Food and Drug Administration (FDA) for in vitro diagnostic use. Performance characteristics of additions to the assay were determined by the UNC Hospitals Molecular Genetics Laboratory.

Three polymorphisms that have the potential to confound mutation analysis are evaluated along with analysis of the intron 8 polypyrimidine tract polymorphism (5T, 7T and 9T). Intron 8 analysis is reported for CF diagnostic tests, and in carrier tests when an R117H mutation is detected because 5T in *cis* with R117H is a disease-related allele. Results are reported as either consistent with a diagnosis of CF (two

CFTR mutations found); at least carrier status (one mutation identified); or no detectable mutation (which reduces the probability of CF or carrier status).

Table 1. 60 mutations tested in UNC Hospitals CF mutation panel. Nomenclature is based on *CFTR* reference sequence NM_000492.3. Legacy names for selected mutations are shown in parentheses.

c.54-5940_273+10250del21kb p.Ser18Argfs (dele2,3)	c.1675G>A, p.Ala559Thr (A559T)
c.178G>T, p.Glu60* (E60X)	**c.1679G>C, p.Arg560Thr (R560T)**
c.223C>T, p.Arg75* (R75X)	**c.1766+1G>A (1898+1G>A)**
c.254G>A, p.Gly85Glu (G85E)	c.1766+5G>T (1898+5G>T)
c.262_263delTT, p.Leu88Ilefs (394delTT)	c.1923_1931del9insA, p.Ser641Argfs (2055del9>A)
c.274-1G>A (406-1G>A)	c.2012delT, p.Leu671* (2143delT)
c.350G>A, p.Arg117His (R117H)	c.2051_2052delAAinsG, p.Lys684Serfs (2183AA>G)
c.366T>A, p.Tyr122* (Y122X)	**c.2052delA, p.Lys684Asnfs (2184delA)**
c.489+1G>T (621+1G>T)	c.2128A>T, p.Lys710* (K710X)
c.532G>A, p.Gly178Arg (G178R)	c.2175_2176insA, p.Glu726Argfs (2307insA)
c.579+1G>T (711+1G>T)	**c.2657+5G>A (2789+5G>A)**
c.617T>G, p.Leu206Trp (L206W)	c.2668C>T, p.Gln890* (Q890X)
c.803delA, p.Asn268Ilefs (935delA)	**c.2988+1G>A (3120+1G>A)**
c.948delT, p.Phe316Leufs (1078delT)	c.3067_3072del, p.Ile1023_Val1024del (3199del6)
c.988G>T, p.Gly330* (G330X)	c.3196C>T, p.Arg1066Cys (R1066C)
c.1000C>T, p.Arg334Trp (R334W)	c.3266G>A, p.Trp1089* (W1089X)
c.1040G>A, p.Arg347His (R347H)	c.3276C>A, p.Tyr1092* (Y1092X)
c.1040G>C, p.Arg347Pro (R347P)	c.3302T>A, p.Met1101Lys (M1101K)
c.1364C>A, p.Ala455Glu (A455E)	c.3454G>C, p.Asp1152His (D1152H)
c.1477C>T, p.Gln493* (Q493X)	c.3472C>T, p.Arg1158* (R1158X)
c.1519_1521delATC, p.Ile507del (Deltal507)	**c.3484C>T, p.Arg1162* (R1162X)**
c.1521_1523delCTT, p.Phe508del (DeltaF508)	**c.3528delC, p.Lys1177Serfs (3659delC)**
c.1545_1546delTA, p.Tyr515* (1677delTA)	c.3587C>G, p.Ser1196* (S1196X)
c.1558G>T, pVal520Phe (V520F)	c.3659delC, p.Thr1220Lysfs (3791delC)
c.1585-1G>A (1717–1G>A)	**c.3717+12191C>T (3849+10kbC>T)**
c.1624G>T, p.Gly542* (G542X)	c.3744delA, p.Lys1250Argfs (3876delA)
c.1646G>A, p.Ser549Asn (S549N)	c.3764C>A, p.Ser1255* (S1255X)
c.1647T>G, p.Ser549Arg (S549R)	c.3773dupT, p.Leu1258Phefs (3905insT)
c.1652G>A, p.Gly551Asp (G551D)	**c.3846G>A, p.Trp1282* (W1282X)**
c.1657C>T, p.Arg553* (R553X)	**c.3909C>G, p.Asn1303Lys (N1303K)**

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 919-966-2229. The spectrum of mutations within each ethnic sub-population corresponds to a different test detection rate for this 60-mutation panel. Approximate carrier rates and residual risks are summarized in Table 2.

Table 2. Classical CF detection rate of the 60 mutation panel is shown according to ethnicity. Also shown are pre- and post-test carrier risks in individuals with a negative family history of cystic fibrosis.

Ethnic Group	Detection Rate	Pre-Test Risk	Post-Test Risk
Ashkenazi Jewish	97%	1/25	1/800
European White	91%	1/25	1/270
Hispanic American	84%	1/46	1/280
African American	73%	1/65	1/240
Asian	55%	1/90	1/200

References:

1. Online Mendelian Inheritance in Man: http://omim.org/entry/219700
2. Gene Reviews: http://www.ncbi.nlm.nih.gov/books/NBK1250/.
3. Watson MS, et al. Cystic fibrosis population carrier screening: 2004 revision of the American College of Medical Genetics mutation panel. Genet Med 2004;6:387-391).

Questions?
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