Cystic Fibrosis Mutation Analysis

Genetics

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which encodes a chloride ion (Cl^-)-selective channel protein that is localized within the outer membrane of epithelial cells of sweat glands, lungs, pancreas, and other exocrine glands. CF-causing mutations may give rise to CFTR proteins that do not fold properly and are ultimately degraded, do not localize to the apical membrane, or are expressed in low amounts. More than 1500 mutations have been described, and different mutations cause different defects in the CFTR protein, sometimes causing a milder or more severe disease. Moreover, various genetic modifiers can modulate the frequency and severity of the disease. The most common mutation is F508del, previously termed ΔF508, which accounts for approximately two thirds of all *CFTR* alleles in patients with CF, with a decreasing prevalence from Northwest to Southeast Europe. The remaining third of alleles are substantially heterogeneous, with fewer than 20 mutations occurring at a worldwide frequency of more than 0.1%. Some mutations can reach a higher frequency in certain populations, due to a founder effect in religious, ethnic or geographical isolates. Although CF is most prevalent in Caucasians (1 in 2,500), it is a panethnic disease in North America, found in all races, including African-American (1 in 15,000), Hispanic-American (1 in 13,500), and Asian-American (1 in 35,000) individuals. This reflects the heterogeneity of the North American population.

Mutations within the *CFTR* gene are classified as “classic” (severe) belonging to classes I, II and III or “non-classic” (mild) belonging to classes IV and V, and in general according to the phenotype typically associated with the mutation (Table 1). CF is caused by the presence of two classic (severe) mutations (homozygous or compound heterozygous) mutations in both alleles of the *CFTR* gene, and is inherited in an autosomal recessive manner. Heterozygous carriers of *CFTR* mutations do not develop CF but exhibit increased risk for pancreatitis and associated pancreatic damage characterized by elevated mucus levels, fibrosis and cyst formation. However, there is considerable clinical heterogeneity associated with individual mutations, and environmental factors and modifier genes play critical roles in determining clinical outcome. For instance, individuals carrying mutations like D1152H together with a CF-causing mutation like F508del may show a clinical spectrum ranging from male infertility due to congenital bilateral absence of the vas deferens (CBAVD) to CF with pancreatic sufficiency but fully expressed lung disease. Also, there are some class I mutations in the *CFTR* gene that encode a stop codon close to the end of the gene, resulting in a functioning CFTR protein and a pancreatic sufficient (PS) phenotype. Also, about 2–3% of CF patients homozygous for classic mutations are PS at diagnosis but most experience gradual transition to pancreatic insufficiency (PI) during childhood or adulthood. CF twin and siblings studies show that the proportion of CF variance that can be attributed to genetic factors (i.e., heritability) ranges from 0.6 to 0.8; therefore, genotype is not a good predictor of clinical outcome and should not be used as an indicator of prognosis.

Test Method

This **Luminex xTAG® Cystic Fibrosis 39 kit v2** is approved by the U.S. Food and Drug Administration to screen for 39 mutations and 4 variants in the *CFTR* gene associated with cystic fibrosis. The panel includes the 23 mutations/variants currently recommended by the American College of Medical Genetics and American College of Obstetricians and Gynecologists (ACMG/ACOG)*, plus 16 other mutations (Table 1). However, over 1500 *CFTR* mutations have been documented worldwide. This panel provides approximate *CFTR* mutation detection rates of 90.5% in North American Caucasians, 73.8% in Hispanic Americans, 67.5% in African Americans, 48.9% in Asian Americans, and 94.0% in Ashkenazi Jews based on reported mutation frequencies in ethnic groups.

Table 1: *CFTR* mutations/variants detected by the Luminex xTAG® Cystic Fibrosis 39 kit v2

Red=severe or classic mutation; Green=mild or non-classic mutation; Blue=variants; *=ACOG recommended panel

Mutation	Comments
G85E*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
R117H*	Consequence depends on associated 5T, 7T, 9T variant; may or may not cause CF when combined with a CF-causing mutation; likely to be PS; second most common mutation in US population
Y122X	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; most prevalent in French Reunion Islanders
R334W*	Causes CF when combined with another CF-causing variant; likely PS; most prevalent in Hispanics
R347H	Causes CF when combined with another CF-causing variant; likely PS
R347P*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
A455E*	Causes CF when combined with another CF-causing variant; likely PS
I507del*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
F508del*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; ~70% CF chromosomes in Northern Europeans but < 50% in Spanish and Hispanics
V520F	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
G542X*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; 2nd most common CF mutation worldwide
S549N	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; most prevalent in Hispanics
S549R	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
G551D*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; some patients PS or PS then convert to PI
R553X*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
A559T	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; most prevalent in African Americans
R560T*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; some patients PS then convert to PI
Y1092X	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
M1101K	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; most common CF mutation in North American Hutterites and uncommon outside
R1162X*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; most prevalent in Native Americans
S1255X	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; most prevalent in African Americans
W1282X*	Causes CF when combined with another CF-causing variant and PI when combined with

Mutation	Comments
	another variant that causes PI; 60% Ashkenazi Jews, 1% Caucasians
N1303K*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
394delTT	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
621+1G>T*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; most prevalent in African Americans
711+1G>T*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; most prevalent in African Americans
1078delT	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
1717-1G>A*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
1898+1G>A*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
1898+5G>T	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; most prevalent in Chinese
2183AA>G	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
2184delA*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; some patients PS then convert to PI
2307insA	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; most prevalent in African Americans
2789+5G>A*	Causes CF when combined with another CF-causing variant; likely PS
3120+1G>A*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; 9-14% of African-American CF chromosomes
3659delC*	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; some patients PS then convert to PI
3849+10kbC>T*	Causes CF when combined with another CF-causing variant; likely PS
3876delA	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI; most prevalent in Hispanics, rare in other ethnic groups
3905insT	Causes CF when combined with another CF-causing variant and PI when combined with another variant that causes PI
5T/7T/9T	Poly-T tract variants that influence effect of R117H
F508C	This variant does not cause CF when combined with another CF-causing variant
I507V	This variant does not cause CF when combined with another CF-causing variant
I506V	This variant does not cause CF when combined with another CF-causing variant

General Limitations of Test: Results of this test are not intended to be used as the sole means for clinical diagnosis or patient management. Substantial clinical variability exists among the different *CFTR* mutations. There is also substantial clinical variability among CF patients with identical genotypes; therefore, it is difficult to predict individual patient outcome on the basis of *CFTR* genotype alone. The use of *CFTR* genotype to predict prognosis in individuals with CF at the time of their diagnosis is not recommended. Patients should receive appropriate genetic counseling to explain the implications of test results, the residual risks and uncertainties, and the reproductive or medical options that they raise for patients and/or family. I506V, I507V and F508C are considered benign polymorphisms but can produce false positive

I507del or F508del allelic calls using this test. Underlying polymorphisms or mutations in primer-binding regions could lead to incorrect assignment of homozygosity.

Homozygous or Compound Heterozygous, Classic (Severe) Mutations: Homozygosity or compound heterozygosity with classic mutations results in classic CF. Parental testing is recommended for compound heterozygotes and for homozygotes. If the two mutations are on the same parental *CFTR* gene, they are said to be in *cis*; if each mutation is on a different parental *CFTR* gene, they are said to be in *trans*. When the two mutations are in *cis*, CF may not be confirmed, and the search of another *CFTR* mutation, located in *trans* with the other two, should be continued. All homozygous mutations detected should be confirmed by sequencing except for I507del and F508del. For I507del and F508del homozygotes, sequence is recommended only if an I506V, I507V, or F508C variant is detected.

Heterozygous, Classic (Severe) Mutation: The presence of one classic mutation in *CFTR* suggests that the individual is a carrier; however, CF cannot be excluded due to the possibility that an additional *CFTR* mutation not targeted by the assay may be present. Absence of a mutation in one or both alleles does not preclude the presence of *CFTR* mutations/variants in the patient's specimen.

R117H Mutation: The R117H mutation is generally considered to be a mild CF (class IV) mutation. When combined with another CF-causing variant it is associated with a broad phenotype, ranging from CF with suppurative lung disease, to no clinical disease. A polythymidine tract, comprising 5(5T), 7(7T), or 9(9T) repeats, in intron 8 of the *CFTR* gene influences CFTR mRNA splicing whereby fewer number of thymidine repeats results in greater skipping of exon 9, and ultimately in greater amounts of aberrant CFTR protein. Generally, in order for the R117H mutation to function as a classic CF mutation, it must occur in *cis* (i.e., on the same chromosome) with the 5T variant, which produces the highest proportion of non-functional CFTR protein. When R117H-5T occurs on one chromosome and is coupled with a classic CF mutation on the other chromosome (e.g., F508del/R117H-5T compound heterozygote), the child may have an elevated or borderline sweat test, mild to moderate lung disease, pancreatitis, and male infertility (i.e., pancreatic sufficient CF). By contrast, when R117H is in *trans* with 5T (or in *cis* or *trans* with 7T or 9T) it acts as a mild CF mutation and when coupled with a classic mutation produces a variable phenotype, including individuals with normal or borderline sweat tests, late-onset, pancreatic sufficient CF, CBAVD alone (esp. F508del/R117H-5T*trans* or F508del/R117H-7T), or no disease at all (esp. F508del/R117H-9T). Testing of the parents of R117H/5T or R117/7T positive compound heterozygote infants can be used to determine *cis* vs *trans* positioning of R117H relative to the 5T or 7T variants. F508del invariably segregates with the 9T allele so testing of parents of F508del/R117H (5T/9T or 7T/9T) compound heterozygote infants is not recommended. The 7T/7T is the most frequent (~80%) genotype associated with R117H in the North American population, followed by 5T/7T and 7T/9T (each ~10%) and 5T/5T, 5T/9T and 9T/9T (each less than 1%). Table 2 indicates some of the clinical interpretations for genotype combinations involving R117H.

Table 2. Clinical interpretations involving R117H, other *CFTR* mutations and the 5T/7T/9T variants

Allele 1	Allele 2	5T,7T,9T Variants	Clinical Interpretation
R117H	None detected	Any combination	Diagnosis of CF unlikely, but cannot exclude
R117H	F508del or other severe CF mutation	5T included	Consistent with diagnosis of CF (when 5T in *cis* with R117H)
R117H	F508del or other severe CF mutation	7T included	Diagnosis of CF possible (when 7T in *cis* with R117H), but variable phenotype
R117H	F508del or other severe CF mutation	9T/9T	Diagnosis of CF unlikely. Possibility of CF-related disorder (e.g., CBAVD in males)
R117H	Mild CF mutation (i.e., non-classical)	5T or 7T included	Variable phenotype from mild to no disease (depends on *cis* or *trans* position of R117H relative to T variant)
R117H	Mild CF mutation (i.e., non-classical)	9T/9T	Diagnosis of CF unlikely, but cannot exclude

No Mutations Detected:
When no mutations are detected for both alleles, the diagnosis of CF is unlikely but cannot be excluded due to the possibility that a *CFTR* mutation not targeted by the assay may be present. The approximate carrier residual risk in an individual following a negative *CFTR* mutation test is as follows:

Ethnicity[a]	**Risk Prior to Testing**	**Approx. Risk After Negative *CFTR* Mutation Analysis**
Ashkenazi Jewish	1/24	1/400
North American Caucasian	1/25	1/208
Hispanic American	1/46	1/164
African American	1/65	1/186
Asian American	1/94	1/184

[a] Risk assessments based on ethnicity assuming no family history of CF[4]

References:

1. Sosnay PR, *et al*. Applying Cystic Fibrosis Transmembrane Conductance Regulator Genetics and $CFTR_2$ Data to Facilitate Diagnoses. *J Pediatrics* 2017; **181**; S27–S32.e1
2. Egan ME. Genetics of Cystic Fibrosis: Clinical Implications. *Clin Chest Med* 2016; **37**(1):9-16.
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4. ACOG Committee Opinion. Update on carrier screening for cystic fibrosis. Obstet Gynecol 2005;106:1465-8.