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Cystic Fibrosis Pathogenic Variant Analysis

Background

Cystic fibrosis (CF) (OMIM: 219700) is a multisystem genetic disease of sodium chloride transport that commonly involves the lungs, pancreas, intestines, liver, sweat glands and male reproductive system. Respiratory failure is the leading cause of death among individuals with CF, with median life expectancy of 46 years (CF Foundation).[1] CF is one of the most common inherited conditions among Caucasians and is diagnosed in approximately 1 in 3,000 individuals.[2-3] The condition is less common but does occur among all other racial groups. CF has an autosomal recessive inheritance pattern and heterozygous carriers are unaffected. If both partners in a couple are CF carriers, there is a 25% chance for each child to have CF and a 50% chance for each child to be a carrier.

The cystic fibrosis transmembrane conductance regulator (*CFTR)* gene was isolated in 1989.[4] The most common pathogenic variant, deltaF508, was identified in the same year and represents 70% of CF pathogenic variants among Caucasians.[5] To date, more than 1,800 gene variants have been found, though most are quite rare.[6] Pathogenic variants in *CFTR* have also been identified in individuals with atypical presentations, such as acute or chronic recurrent pancreatitis and isolated congenital absence of vas deferens (CAVD), as well as in individuals with late-onset or mild CF symptoms.[7]

Clinical Indications

Carrier Screening

In order to identify couples at risk of having a child with CF, both the American College of Medical Genetics and the American College of Obstetricians and Gynecologists recommend that CF carrier screening be offered routinely to women of reproductive age.[8] Screening should include the pan-ethnic panel of the 23 most common *CFTR* pathogenic variants.

Diagnostic Testing

In both pediatric and adult patients in whom *CFTR* related disorders are suspected, genetic testing is routinely performed as an initial diagnostic test or as confirmation of clinical findings or sweat chloride testing, which remains the standard diagnostic test.[9]

Newborn screening (NBS) for cystic fibrosis is now offered universally in the United States through each state's mandatory NBS program.[10] Initial screening measures a digestive enzyme, immunoreactive trypsinogen (IRT). Because elevations in IRT may have other causes, a *CFTR* variant analysis is often performed as a secondary screen, though this varies by state. Since the advent of routine carrier screening and NBS, many individuals with CF are recognized at, or even before, birth.

Methodology

DNA samples are amplified in a multiplex reaction for regions of interest in *CFTR*. Initial amplification is followed by single base extension (SBE) reactions to detect known *CFTR* variants. The extension products are analyzed using matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometry. Multiple variants may be detected because each *CFTR* variant assay contains a primer of unique length and mass, allowing specific genotypes to be assigned.

Mutations Detected

The variants included on the Cystic Fibrosis Pathogenic Variant Analysis assay represent clinically validated variants classified as CF-causing in the CFTR2 database at Johns Hopkins University, a product of the CFTR2 (Clinical and Functional Translation of *CFTR*) initiative. The assay tests for: 140 CF-causing variants; one variant (F508C) associated with Congenital Absence of the Vas Deferens (CAVD); one ACMG-recommended panel variant (R117H) classified as a Mutation of Varying Clinical Consequence by CFTR2; one conditionally reported modifying variant (Poly T); and two conditionally reported benign variants (I506V and I507V). This panel includes the 23 pathogenic variants recommended for universal carrier screening by the American College of Medical Genetics. The panel also encompasses all variants that are detected by the Ohio newborn screen.

Interpretation

Results should be used and interpreted in the context of clinical evaluation. This test does not detect all variants in the *CFTR* gene and it is possible that this individual could have a *CFTR* variant not included in this test. Therefore, the failure to identify a variant does not guarantee that other *CFTR* variants are not present in the sample being analyzed. Variants identified by this assay vary in frequency among different populations.[9,11] Residual CF carrier risk following a negative Cystic Fibrosis Pathogenic Variant Analysis result is based on ethnicity and personal/family history.

Residual Cystic Fibrosis Carrier Risk after Negative Carrier Screen
(Residual risk for unlisted ethnicities is unknown.)

Ethnicity	Carrier rate	Detection rate (%)	Residual carrier risk (with negative family history)
African American	1 in 61	74	1 in 231
Ashkenazi Jewish	1 in 24	97	1 in 768
Asian	1 in 94	49	1 in 183
Caucasian	1 in 25	91	1 in 267
Hispanic	1 in 58	77	1 in 248

As with any hybridization-based assay, underlying gene variants in oligonucleotide-binding regions can affect the alleles being probed and, consequently, the calls made. The orientation of the Poly T variant, whether in cis/trans to the R117H variant, cannot be ascertained via this assay.

Due to the complex issues surrounding cystic fibrosis, informed consent and genetic counseling are recommended for all individuals undergoing testing.

References

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Additional Resources

Ong T, Marshall SG, Karczeski BA, Sternen DL, Cheng E, Cutting GR. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. Cystic Fibrosis and Congenital Absence of the Vas Deferens. 2001 Mar 26 [Updated 2017 Feb 2].

Available from: https://www.ncbi.nlm.nih.gov/books/NBK1250/

Cystic fibrosis (CFTR RefSeq# NM_000492.3)
Legacy names of 142 pathogenic variants with 3 *conditionally reported variants*

1078delT	2585delT	663delT	G178R	Q552X	S489X
1154insTC	2622+1G>A	711+1G>T	G330X	Q890X	S492F
1213delT	2711delT	711+3A>G	G542X	Q98X	S549N
1248+1G>A	2789+5G>A	711+5G>A	G551D	R1066C	S549R-CGT
1259insA	394delTT	712-1G>T	G85E	R1066H	S549R-AGG
1341+1G>A	3007delG	852del22	G970R	R1158X	S945L
1461ins4	3120+1G>A	935delA	H199Y	R1162X	T338I
1525-1G>A	3120G>A	A455E	I336K	R117C	V520F
1548delG	3121-1G>A	A559T	K710X	R117H	W1089X
1677delTA	3199del6	CFTRdele2,3	L1065P	R334W	W1204X (3611)
1717-1G>A	3272-26A>G	CFTRdele22,23	L1077P	R347H	W1204X (3612)
1717-8G>A	3659delC	D110H	L206W	R347P	W1282X
1811+1.6kbA>G	3791delC	D1152H	L467P	R352Q	W401X
1812-1G>A	3849+10KbC>T	deltaF508	L732X	R553X	W846X
1898+1G>A	3876delA	deltaI507	L927P	R560K	Y1092X
1898+3A>G	3905insT	E1104X	M1101K	R560T	Y1092X
1898+5G>A	405+1G>A	E585X	M1V	R709X	Y122X
1898+5G>T	406-1G>A	E60X	N1303K	R75X	*Poly T*
2055del9>A	4005+1G>A	E822X	P205S	R764X	*I506V*
2143delT	4016insT	E831X	P67L	R851X	*I507V*
2183AAtoG	4209TGTT>AA	E92K	Q1313X	S1196X	
2184delA	4382delA	E92X	Q220X	S1251N	
2184insA	457TAT>G	F508C	Q39X	S1255X	
2307insA	574delA	G1244E	Q493X	S341P	
2347delG	621+1G>T	G1349D	Q525X	S466X	

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Test Overview

Test Name	Cystic Fibrosis Pathogenic Variant Analysis
Ordering Mnemonic	CFMDX
Specimen	Peripheral Blood: 4 ml in an EDTA tube (lavender top)
Stability	Ambient: 24 hours. Frozen: Unacceptable. Refrigerated: 5 days.
Reference Range	No variant detected.
CPT Code	81220

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