Cystic Fibrosis (*CFTR*), 165 Pathogenic Variants with Reflex to Sequencing and Reflex to Deletion/Duplication

Indications for Ordering

For individuals with suspected cystic fibrosis (CF) or a *CFTR*-related disorder

Test Description

- Polymerase chain reaction followed by genotyping with hydrolysis probes targeting 165 pathogenic *CFTR* gene variants (see table)
- Bidirectional sequencing of the *CFTR* coding regions and intron/exon boundaries
- Deletion/duplication analysis by multiplex ligation-dependent probe amplification

Tests to Consider

Primary tests

Cystic Fibrosis (*CFTR*) 165 Pathogenic Variants with Reflex to Sequencing and Reflex to Deletion/Duplication 2013664

- For individuals with suspected CF
- This test is NOT indicated for routine obstetric carrier screening
- If individual is not symptomatic, order the CF 165 pathogenic variants test

Cystic Fibrosis (*CFTR*) 165 Pathogenic Variants with Reflex to Sequencing 2013663

- For individuals with suspected CF
- This test is NOT indicated for routine obstetric carrier screening
- If individual is not symptomatic, order the CF 165 pathogenic variants test

Related tests

Cystic Fibrosis (*CFTR*) 165 Pathogenic Variants 2013661

- Carrier screening for expectant individuals and those planning a pregnancy
- Diagnostic testing for individuals with symptoms of classic CF

Cystic Fibrosis (*CFTR*) Sequencing 0051110

- For individuals with suspected CF but without 2 pathogenic variants detected by the CF 165 pathogenic variants test

Cystic Fibrosis (*CFTR*) Sequencing with Reflex to Deletion/Duplication 0051640

- For individuals with suspected CF but without 2 pathogenic variants detected by the CF 165 pathogenic variants test

Familial Mutation, Targeted Sequencing 2001961

- Useful when a pathogenic familial variant identifiable by sequencing is known

Disease Overview

Incidence

- Classic CF (Abeliovich, 1992)
  - Ashkenazi Jews – 1/2,300
  - Caucasians – 1/2,500
  - Hispanic Americans – 1/13,500
  - African Americans – 1/15,100
  - Asian Americans – 1/35,100
- Other *CFTR*-related disorders – unknown

Symptoms

- Classic CF
  - Chronic sinopulmonary disease
  - Gastrointestinal/nutritional abnormalities
    - Newborns may have meconium ileus and/or failure to thrive
  - Obstructive azoospermia
  - Salt loss syndromes
  - Life expectancy – ~38 years
- Other *CFTR*-related disorders
  - Variable symptoms
    - Idiopathic pancreatitis
    - Congenital bilateral absence of the vas deferens (CBAVD)
    - Bronchiectasis
    - Nasal polyposis
  - Typically presents in adulthood
    - May not decrease life expectancy

Genetics

Gene – *CFTR*

Inheritance – autosomal recessive

Penetrance

- Severe pathogenic variants – high
- Mild/moderately severe pathogenic variants – variable

Variants

- >2,000 variants in *CFTR* gene
  - Most are very rare and not well characterized
  - 2.6% are large insertions/deletions
  - *CFTR* is the only gene known to be causative for CF
- Men with CBAVD
  - At least one pathogenic *CFTR* variant – ~75%
    - Two pathogenic *CFTR* variants – ~20%
    - One pathogenic *CFTR* variant and one 5T variant – 25%
    - One pathogenic *CFTR* variant – 20%
    - One 5T variant – 10%
- Individuals with idiopathic pancreatitis
  - Up to 40% are predicted to have at least one pathogenic *CFTR* variant
- Purulent pansinusitis or nasal polyposis starting early in life or associated with chronic infection
  - 30% of adults have one pathogenic *CFTR* variant
  - 7% of adults have two pathogenic *CFTR* variants

Test Interpretation

Sensitivity/specificity

- Clinical sensitivity – 99%
  - Sequencing – 97% (Strom, 2003)
  - Deletion/duplication – 2.5% (Cystic Fibrosis Mutation Database, 2011)
- Analytical sensitivity/specificity
  - Sequencing – 99%
  - MLPA – 90%

Results

- Two severe pathogenic *CFTR* variants on opposite chromosomes
  - Predicted to be affected with classic CF
- One severe and one moderately severe CF-causing variant on opposite chromosomes
  - Predicted to be affected with pancreatic sufficient CF
- One mild pathogenic non CF-causing variant in combination with a CF-causing variant or another mild non-CF causing variant on the opposite chromosome
  - Increased risk for a *CFTR*-related disorder such as pancreatitis, CBAVD, and respiratory disease
- Only one severe or moderately severe variant
  - At least a CF carrier, but may be affected if a promoter or deep intronic variant is present that was not identified
- No pathogenic variants identified
  - Unlikely to be either affected with, or a carrier of, CF

Limitations

- Diagnostic errors can occur due to rare sequence variations
- Not detected
  - Breakpoints of large deletions/duplications
  - Regulatory region and deep intronic variants
- *CFTR* gene sequencing or deletion/duplication testing may identify variants of unknown clinical significance

References

- Abeliovich D, Lavon IP, et al. Screening for five mutations detects 97% of cystic fibrosis (CF) chromosomes and predicts a carrier frequency of 1:29 in the Jewish Ashkenazi population. Am J Hum Genet. 1992;51(5): 951-956
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- Heim RA, Sugarman EA, et al. Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation test. Genet Med. 2001;3:168-176
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- Sugarman EA, Rohlfs EM, et al. *CFTR* mutation distribution among U.S. Hispanic and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. Genet Med. 2004;6:392-399
- Update on carrier screening for cystic fibrosis. Committee Opinion No. 486. American College of Obstetricians and Gynecologists. Obstet Gynecol. 2011;117:1028-1031
Watson MS, Cutting GR, et al. Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. Genet Med. 2004;6(5):387-391

***CFTR* 165 Pathogenic Variants Tested**		
Legacy Name	**cDNA Name**	**Protein Name**
M1V	c.1A>G	p.Met1Val
P5L	c.14C>T	p.Pro5Leu
CFTRdele2,3 (deletion of exons 2 and 3)	c.54-5940_273+10250del21kb	p.Ser18ArgfsX16del
Q39X	c.115C>T	p.Gln39X
E60X	c.178G>T	p.Glu60X

Legacy Name	cDNA Name	Protein Name
P67L	c.200C>T	p.Pro67Leu
R75X	c.223C>T	p.Arg75X
➢ **G85E**	**c.254G>A**	**p.Gly85Glu**
394delTT	c.262_263delTT	p.Leu88IlefsX22
405+1G>A	c.273+1G>A	Intronic
406-1G>A	c.274-1G>A	Intronic
E92K	c.274G>A	p.Glu92Lys
E92X	c.274G>T	p.Glu92X
Q98X	c.292C>T	p.Gln98X
444delA	c.313delA	p.Ile105SerfsX2
457TAT>G	c.325_327delTATinsG	p.Tyr109GlyfsX4
D110H	c.328G>C	p.Asp110His
R117C	c.349C>T	p.Arg117Cys
➢ **R117H**	**c.350G>A**	**p.Arg117His**
Y122X	c.366T>A	p.Tyr122X
574delA	c.442delA	p.Ile148LeufsX5
➢ **621+1G>T**	**c.489+1G>T**	**Intronic**
R170H	c.509G>A	p.Arg170His
663delT	c.531delT	p.Ile177MetfsX12
G178R	c.532G>A	p.Gly178Arg
➢ **711+1G>T**	**c.579+1G>T**	**Intronic**
711+5G>A	c.579+5G>A	Intronic
711+3A>G	c.579+3A>G	Intronic
712-1G>T	c.580-1G>T	Intronic
H199Y	c.595C>T	p.His199Tyr
P205S	c.613C>T	p.Pro205Ser
L206W	c.617T>G	p.Leu206Trp
Q220X	c.658C>T	p.Gln220X
852del22	c.720_741delAGGGAGAATGATGATGAAGTAC	p.Gly241GlufsX13
935delA	c.803delA	p.Asn268IlefsX17
F311del	c.933_935delCTT	p.Phe312del
1078delT	c.948delT	p.Phe316LeufsX12
G330X	c.988G>T	p.Gly330X
➢ **R334W**	**c.1000C>T**	**p.Arg334Trp**
R334L	c.1001G>T	p.Arg334Leu
I336K	c.1007T>A	p.Ile336Lys
S341P	c.1021T>C	p.Ser341Pro
1154insTC	c.1022_1023insTC	p.Phe342HisfsX28
R347H	c.1040G>A	p.Arg347His
➢ **R347P**	**c.1040G>C**	**p.Arg347Pro**
T351S	c.1052C>G	p.Thr351Ser
R352W	c.1054C>T	p.Arg352Trp
R352Q	c.1055G>A	p.Arg352Gln
1213delT	c.1081delT	p.Trp361GlyfsX8
1248+1G>A	c.1116+1G>A	Intronic
1259insA	c.1127_1128insA	p.Gln378AlafsX4
1288insTA	c.1153_1154insAT	p.Asn386IlefsX3

Legacy Name	cDNA Name	Protein Name
W401X(TAG)	c.1202G>A	p.Trp401X
W401X(TGA)	c.1203G>A	p.Trp401X
1341+1G>A	c.1209+1G>A	Intronic
IVS8 5T[1]	c.1210−12[5]	Intronic
1461ins4	c.1329_1330insAGAT	p.Ile444ArgfsX3
➢ **A455E**	**c.1364C>A**	**p.Ala455Glu**
1525-1G>A	c.1393-1G>A	Intronic
S466X(TAA)	c.1397C>A	p.Ser466X
S466X(TAG)	c.1397C>G	p.Ser466X
L467P	c.1400T>C	p.Leu467Pro
1548delG	c.1418delG	p.Gly473GlufsX54
G480C	c.1438G>T	p.Gly480Cys
S489X	c.1466C>A	p.Ser489X
S492F	c.1475C>T	p.Ser492Phe
Q493X	c.1477C>T	p.Gln493X
W496G	c.1486T>G	p.Trp496Gly
➢ **I507del**	**c.1519_1521delATC**	**p.Ile507del**
➢ **F508del**	**c.1521_1523delCTT**	**p.Phe508del**
1677delTA	c.1545_1546delTA	p.Tyr515X
V520F	c.1558G>T	p.Val520Phe
C524X	c.1572C>A	p.Cys524X
Q525X	c.1573C>T	p.Gln525X
➢ **1717-1G>A**	**c.1585-1G>A**	**Intronic**
1717-8G>A	c.1585-8G>A	Intronic
➢ **G542X**	**c.1624G>T**	**p.Gly542X**
S549R(A>C)	c.1645A>C	p.Ser549Arg
S549N	c.1646G>A	p.Ser549Asn
S549R(T>G)	c.1647T>G	p.Ser549Arg
G551S	c.1651G>A	p.Gly551Ser
➢ **G551D**	**c.1652G>A**	**p.Gly551Asp**
Q552X	c.1654C>T	p.Gln552X
➢ **R553X**	**c.1657C>T**	**p.Arg553X**
A559T	c.1675G>A	p.Ala559Thr
R560G	c.1678A>G	p.Arg560Gly
R560K	c.1679G>A	p.Arg560Lys
➢ **R560T**	**c.1679G>C**	**p.Arg560Thr**
1811+1.6kbA>G	c.1679+1.6kbA>G	Intronic
1812-1G>A	c.1680-1G>A	Intronic
1833delT	c.1703delT	p.Leu568CysfsX4
P574H	c.1721C>A	p.Pro574His
E585X	c.1753G>T	p.Glu585X
➢ **1898+1G>A**	**c.1766+1G>A**	**Intronic**
1898+3A>G	c.1766+3A>G	Intronic
1924del7	c.1792_1798delAAAACTA	p.Lys598GlyfsX11
2055del9>A	c.1923_1931del9insA	p.Ser641ArgfsX5
2105-2117del13insAGAAA	c.1973_1985del13insAGAAA	p.Arg658LysfsX4
2143delT	c.2012delT	p.Leu671X

Legacy Name	cDNA Name	Protein Name
2183delAA	c.2051_2052delAA	p.Lys684ThrfsX4
2183AA>G	c.2051_2052delAAinsG	p.Lys684SerfsX38
➢ **2184delA**	**c.2052delA**	**p.Lys684AsnfsX38**
R709X	c.2125C>T	p.Arg709X
K710X	c.2128A>T	p.Lys710X
2307insA	c.2175_2176insA	p.Glu726ArgfsX4
L732X	c.2195T>G	p.Leu732X
2347delG	c.2215delG	p.Val739TyrfsX16
R764X	c.2290C>T	p.Arg764Ter
2585delT	c.2453delT	p.Leu818TrpfsX3
E822X	c.2464G>T	p.Glu822X
2622+1G>A	c.2490+1G>A	Intronic
E831X	c.2491G>T	p.Glu831X
D836Y	c.2506G>T	p.Asp836Tyr
W846X	c.2537G>A	p.Trp846X
R851X	c.2551C>T	p.Arg851X
2711delT	c.2583delT	p.Phe861LeufsX3
➢ **2789+5G>A**	**c.2657+5G>A**	**Intronic**
Q890X	c.2668C>T	p.Gln890X
2869insG	c.2737_2738insG	p.Tyr913X
L927P	c.2780T>C	p.Leu927Pro
2942insT	c.2810_2811insT	p.Val938GlyfsX37
S945L	c.2834C>T	p.Ser945Leu
3007delG	c.2875delG	p.Ala959HisfsX9
L967S	c.2900T>C	p.Leu967Ser
G970R	c.2908G>C	p.Gly970Arg
➢ **3120+1G>A**	**c.2988+1G>A**	**Intronic**
3120G>A	c.2988G>A	Intronic
3121-1G>A	c.2989-1G>A	Intronic
P1013H	c.3038C>A	p.Pro1013His
3171delC	c.3039delC	p.Tyr1014ThrfsX9
3199del6	c.3067_3072delATAGTG	p.Ile1023_Val1024del
3272-26A>G	c.3140-26A>G	Intronic
L1065P	c.3194T>C	p.Leu1065Pro
R1066C	c.3196C>T	p.Arg1066Cys
R1066H	c.3197G>A	p.Arg1066His
L1077P	c.3230T>C	p.Leu1077Pro
W1089X	c.3266G>A	p.Trp1089X
Y1092X(C>A)	c.3276C>A	p.Tyr1092X
Y1092X(C>G)	c.3276C>G	p.Tyr1092X
c.3297C>A	c.3297C>A	p.Phe1099Leu
M1101K	c.3302T>A	p.Met1101Lys
E1104X	c.3310G>T	p.Glu1104X
R1158X	c.3472C>T	p.Arg1158X
➢ **R1162X**	**c.3484C>T**	**p.Arg1162X**
➢ **3659delC**	**c.3528delC**	**p.Lys1177SerfsX15**
S1196X	c.3587C>G	p.Ser1196X

Legacy Name	cDNA Name	Protein Name
W1204X(3743G>A)	c.3611G>A	p.Trp1204X
W1204X(3744G>A)	c.3612G>A	p.Trp1204X
3791delC	c.3659delC	p.Thr1220LysfsX8
➢ **3849+10kbC>T**	**c.3717+12191C>T**	**Intronic**
G1244E	c.3731G>A	p.Gly1244Glu
3876delA	c.3744delA	p.Lys1250ArgfsX9
S1251N	c.3752G>A	p.Ser1251Asn
S1255P	c.3763T>C	p.Ser1255Pro
S1255X	c.3764C>A	p.Ser1255X
3905insT	c.3773_3774insT	p.Leu1258PhefsX7
➢ **W1282X**	**c.3846G>A**	**p.Trp1282X**
4005+1G>A	c.3873+1G>A	Intronic
➢ **N1303K**	**c.3909C>G**	**p.Asn1303Lys**
Q1313X	c.3937C>T	p.Gln1313X
CFTRdele22,23	c.3964-78_4242+577del	p.Exon22-23del
G1343Afs	c.4028delG	p.Gly1343AlafsX4
G1349D	c.4046G>A	p.Gly1349Asp
4209TGTT>AA	c.4077_4080delTGTTinsAA	p.Val1360delfsX3
E1371X	c.4111G>T	p.Glu1371X
4382delA	c.4251delA	p.Glu1418ArgfsX14

➢ **23 variants recommended for carrier screening by ACMG/ACOG**

[1]The IVS8 5T variant, c.1210-12[5], will be reported when R117H is detected and in individuals who are reported to be symptomatic