See below for Additional Technical Information topics

Cystic Fibrosis (*CFTR*) 165 Pathogenic Variants
Fragile X Syndrome
Spinal Muscular Atrophy

Cystic Fibrosis (*CFTR*) 165 Pathogenic Variants

Indications for Ordering

- Carrier screening
  - Expectant couples
  - Couples planning a pregnancy
  - Individuals with a family history of cystic fibrosis (CF)
- Diagnostic testing for individuals with symptoms of CF

Test Description

Polymerase chain reaction followed by fluorescence monitoring of 165 pathogenic *CFTR* gene variants (see Table 1)

- If both the R117H variant and the 5T variant are detected, test will automatically reflex to cis/trans testing to determine whether the variants are on the same chromosome
  - The mild 5T variant, c.1210−12[5], will only be reported if either the R117H variant is detected or the individual is reported to be symptomatic

Tests to Consider

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

Related tests

Cystic Fibrosis (*CFTR*) Sequencing 0051110
- For individuals with suspected CF but without 2 pathogenic variants detected by the CF 165 pathogenic variants test
- This test is NOT indicated for routine obstetric carrier screening

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

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
- This test is NOT indicated for routine obstetric carrier screening

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, Fetal 2013662
- For fetal testing when both parents are known carriers of one of the variants on the CF 165 pathogenic variants test or fetus has an echogenic bowel

Genetic Carrier Screen (CF, FXS, and SMA) with Reflex to Methylation 3000258
- Screen for genetic variants that indicate carrier status for cystic fibrosis (CF), fragile X syndrome (FXS), and spinal muscular atrophy (SMA) in pregnant couples or those planning a pregnancy
- Do not use for diagnostic testing in patients with symptoms of CF, FXS, or SMA

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

Carrier frequency

- Ashkenazi Jews – 1/24
- European Caucasians – 1/25
- Hispanic Americans – 1/58
- African Americans – 1/61
- Asian Americans – 1/94

Symptoms

- Classic CF
  - Chronic sinopulmonary disease and infections
  - Pancreatic insufficiency (endocrine and exocrine)
  - Hepatic disease-biliary obstruction and portal fibrosis
  - Prolapsed rectum
  - Failure to thrive
  - Meconium ileus
  - Obstructive azoospermia
  - Salt loss syndromes
  - Life expectancy – ~41 years
- *CFTR*-related disorders
  - Idiopathic pancreatitis
  - Bilateral absence of the vas deferens (BAVD)
  - Bronchiectasis
  - Nasal polyposis
  - Typically presents in adulthood
    - Often does not decrease life expectancy

Consensus criteria

- The American College of Medical Genetics has recommended all couples planning a pregnancy be offered carrier screening for 23 specific pathogenic *CFTR* variants (Watson, 2004)
- The American Congress of Obstetricians and Gynecologists recommends screening for 23 pathogenic *CFTR* variants in expectant couples (2011)

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
  - CF 165 pathogenic variants test includes the 23 ACMG recommended variants and an additional 142 pathogenic variants (see Table 1)
- Classic CF
  - Two severe or one severe and one moderately severe pathogenic *CFTR* variants on opposite chromosomes
- *CFTR*-related disorders
  - Typically one severe and one mild *CFTR* variant on opposite chromosomes
- BAVD
  - 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%
- Idiopathic pancreatitis
  - Up to 40% 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 is based on ethnicity
  - Ashkenazi Jews – 96%
  - Caucasians – 92%
  - Hispanic Americans – 80%
  - African Americans – 78%
  - Asian Americans – 55%
- Analytical sensitivity/specificity – 99%

Results

- Asymptomatic individuals undergoing carrier screening
  - No detectable variants
    - Reduced CF carrier risk
    - A table with risk reduction based on ethnicity is provided to predict carrier risk (see Table 2)
    - If an individual with a family history of CF has no detectable variants, Bayesian analysis is necessary to determine residual carrier risk
  - One pathogenic variant identified
    - Predicted to be a CF carrier
    - CF screening should be offered to the reproductive partner
- Symptomatic individuals
  - Two severe pathogenic variants or one severe and one moderately severe variant identified
    - Predicted to be affected with pancreatic-insufficient CF and pancreatic-sufficient CF, respectively
  - One severe and one mild pathogenic variant identified
    - Predicted to be at risk for a *CFTR*-related disorder
    - One severe or moderately severe pathogenic variant identified
    - At least a CF carrier
      - Consider *CFTR* gene sequencing and deletion/duplication analysis
    - A table showing the percentage of affected individuals by ethnicity without two detectable pathogenic variants is provided (see Table 3)
  - No detectable variants
    - Decreased risk to be a carrier of or affected with CF
      - Consider *CFTR* sequencing and deletion/duplication testing if suspicion for CF remains
    - A table showing the percentage of affected individuals by ethnicity with no detectable pathogenic variants is provided (see Table 3)

Limitations

- Diagnostic errors can occur due to rare sequence variations
- Only the 165 *CFTR* variants listed will be interrogated

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;51:951-956
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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(3):168-176
- Moskowitz SM, Chmiel JF, et al. Clinical practice and genetic counseling for cystic fibrosis and CFTR-related disorders. Genet Med. 2008 Dec;10(12):851-68
- Sugarman E, 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(5):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
CFTRdele2,3 (deletion of exons 2 and 3)	c.54-5940_273+10250del	Exons 2-3del
Q39X	c.115C>T	p.Gln39X
E60X	c.178G>T	p.Glu60X
P67L	c.200C>T	p.Pro67Leu
R75X	c.223C>T	p.Arg75X
➢ **G85E**	**c.254G>A**	**p.Gly85Glu**
394delTT	c.262_263delTT	p.Leu88IlefsX22 aka p.Leu88fs
405+1G>A	c.273+1G>A	**Intronic**
405+3A>C	C.273+3A>C	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 aka p.Ile105fs
457TAT>G	c.325_327delTATinsG	p.Tyr109GlyfsX4 aka p.Tyr109fs
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 aka p.Ile148fs
➢ **621+1G>T**	**c.489+1G>T**	**Intronic**
663delT	c.531delT	p.Ile177MetfsX12 aka p.Ile177fs
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

CFTR 165 Pathogenic Variants Tested		
Legacy Name	**cDNA Name**	**Protein Name**
H199Y	c.595C>T	p.His199Tyr
P205S	c.613C>T	p.Pro205Ser
L206W	c.617T>G	p.Leu206Trp
Q220X	c.658C>T	p.Gln220X
L227R	c.680T>G	p.Leu227Arg
852del22	c.720_741delAGGGAGAATGATGATGAAGTAC	p.Gly241GlufsX13 aka p.Gly241fs
935delA	c.803delA	p.Asn268IlefsX17 aka p.Asn268fs
936delTA	c.805_806delAT	p.Ile269ProfsX4 aka p.Ile269fs
F311del	c.933_935delCTT	p.Phe312del
1078delT	c.948delT	p.Phe316LeufsX12 aka p.Phe316fs
G330X	c.988G>T	p.Gly330X
➢ **R334W**	**c.1000C>T**	**p.Arg334Trp**
I336K	c.1007T>A	p.Ile336Lys
S341P	c.1021T>C	p.Ser341Pro
1154insTC	c.1022_1023insTC	p.Phe342HisfsX28 aka p.Phe342fs
R347H	c.1040G>A	p.Arg347His
➢ **R347P**	**c.1040G>C**	**p.Arg347Pro**
R352Q	c.1055G>A	p.Arg352Gln
1213delT	c.1081delT	p.Trp361GlyfsX8 aka p.Trp361fs
1248+1G>A	c.1116+1G>A	Intronic
1259insA	c.1127_1128insA	p.Gln378AlafsX4 aka p.Gln378fs
1288insTA	c.1153_1154insAT	p.Asn386IlefsX3 aka p.Asn386fs
W401X(TAG)	c.1202G>A	p.Trp401X
W401X(TGA)	c.1203G>A	p.Trp401X
1341+1G>A	c.1209+1G>A	Intronic
IVS8 5T[a]	c.1210−12[5]	Intronic
1461ins4	c.1329_1330insAGAT	p.Ile444ArgfsX3 aka p.Ile444fs
1471delA	c.1340delA	p.Lys447ArgfsX2 aka p.Lys447fs
➢ **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 aka p.Gly473fs
G480C	c.1438G>T	p.Gly480Cys
S489X	c.1466C>A	p.Ser489X
S492F	c.1475C>T	p.Ser492Phe
Q493X	c.1477C>T	p.Gln493X
➢ **I507del**	**c.1519_1521delATC**	**p.Ile507del**

CFTR 165 Pathogenic Variants Tested		
Legacy Name	**cDNA Name**	**Protein Name**
➢ **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
R560K	c.1679G>A	p.Arg560Lys
➢ **R560T**	**c.1679G>C**	**p.Arg560Thr**
1811+1.6kbA>G	c.1679+1.6kbA>G aka c.1679+1.6kbAG	Intronic
1812-1G>A	c.1680-1G>A	Intronic
1833delT	c.1703delT	p.Leu568CysfsX4 aka p.Leu568fs
Y569D	c.1705T>G	p.Tyr569Asp
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 aka p.Lys598fs
2043delG	c.1911delG	p.Gln637HisfsX26 aka p.Gln637fs
2055del9>A	c.1923_1931del9insA	p.Ser641ArgfsX5 aka p.Ser641fs
2105-2117del13insAGAAA	c.1973_1985del13insAGAAA	p.Arg658LysfsX4 aka p.Arg658fs
2108delA	c.1976delA	p.Asn659IlefsX4 aka p.Asn659fs
2143delT	c.2012delT	p.Leu671X
2183delAA	c.2051_2052del	p.Lys684ThrfsX4
2183AA>G	c.2051_2052delinsG aka c.2051_2delinsG	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 aka p.Glu726fs
L732X	c.2195T>G	p.Leu732X

CFTR 165 Pathogenic Variants Tested		
Legacy Name	**cDNA Name**	**Protein Name**
2347delG	c.2215delG	p.Val739TyrfsX16 aka p.Val739fs
R764X	c.2290C>T	p.Arg764Ter
2585delT	c.2453delT	p.Leu818TrpfsX3 aka p.Leu818fs
E822X	c.2464G>T	p.Glu822X
2622+1G>A	c.2490+1G>A	Intronic
E831X	c.2491G>T	p.Glu831X
W846X	c.2537G>A	p.Trp846X
W846X(2670TGG>TGA	c.2538G>A	p.Trp846X
R851X	c.2551C>T	p.Arg851X
2711delT	c.2583delT	p.Phe861LeufsX3 aka p.Phe861fs
➢ **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 aka p.Val938fs
S945L	c.2834C>T	p.Ser945Leu
3007delG	c.2875delG	p.Ala959HisfsX9 aka p.Ala959fs
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
3171delC	c.3039delC	p.Tyr1014ThrfsX9 aka p.Tyr1014fs
3199del6	c.3067_3072delATAGTG	p.Ile1023_Val1024del aka I1023_V1024del
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
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** **aka p.Lys1177fs**
3667del4	c.3536_3539del	p.Thr1179AsnfsX12 aka p.Thr1179fs
S1196X	c.3587C>G	p.Ser1196X
W1204X(3743G>A)	c.3611G>A	p.Trp1204X
W1204X(3744G>A)	c.3612G>A	p.Trp1204X

CFTR 165 Pathogenic Variants Tested		
Legacy Name	**cDNA Name**	**Protein Name**
3791delC	c.3659delC	p.Thr1220LysfsX8 aka p.Thr1220fs
3821delT	c.3691delT	p.Ser1231ProfsX4 aka p.Ser1231fs
Q1238X	c.3712C>T	p.Gln1238X
➢ **3849+10kbC>T**	**c.3718-2477C>T**	**Intronic**
G1244E	c.3731G>A	p.Gly1244Glu
3876delA	c.3744delA	p.Lys1250ArgfsX9 aka p.Lys1250fs
S1251N	c.3752G>A	p.Ser1251Asn
S1255P	c.3763T>C	p.Ser1255Pro
S1255X	c.3764C>A	p.Ser1255X
3905insT	c.3773_3774insT	p.Leu1258PhefsX7 aka p.Leu1258fs
➢ **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	Exons 22-23del
G1343Afs	c.4028delG	p.Gly1343AlafsX4 aka p.Gly1343fs
G1349D	c.4046G>A	p.Gly1349Asp
4209TGTT>AA	c.4077_4080delTGTTinsAA	p.Val1360delfsX3 aka p.Val1360fs
E1371X	c.4111G>T	p.Glu1371X
4382delA	c.4251delA	p.Glu1418ArgfsX14 aka p.Glu1418fs
➢ **23 variants recommended for carrier screening by ACMG/ACOG** [a]The IVS8 5T variant, c.1210-12[5], will be reported when R117H is detected and in individuals who are reported to be symptomatic		

Table 2. Carrier Risk for Asymptomatic Individuals Before and After a Negative CF 165 Variant Test			
Ethnicity	**Variant Detection Rate**	**Carrier Risk Before Test**	**Carrier Risk After Negative Test**
African Americans	78%	1/61	1/275
Ashkenazi Jews	96%	1/24	1/575
Asian Americans	55%	1/94	1/210
Caucasians	92%	1/25	1/300
Hispanic Americans	80%	1/58	1/285

Table 3. Percentage of Patients with CF who have None or Only One Detectable Variant on the CF 165 Variant Test		
Ethnicity	**CF Patients with No Detectable Pathogenic Variants**	**CF Patients with Only One Detectable Pathogenic Variant**
African Americans	5%	34%
Ashkenazi Jews	1%	7%
Asian Americans	20%	50%
Caucasians	1%	15%
Hispanic Americans	4%	32%

Fragile X Syndrome

Indications for Ordering

- Newborn screening
- Carrier screening for expectant women or those planning a pregnancy
- Individuals with unexplained
  - Intellectual disability
  - Developmental delay
  - Autism
  - Late onset cerebellar ataxia and intention tremor
- Females with
  - Primary ovarian insufficiency (POI)
  - Infertility associated with elevated follicle-stimulating hormone (FSH) levels
  - Family history of fragile X syndrome (FXS) or intellectual disability of unknown etiology
- Prenatal testing for women who carry a fragile X premutation or full mutation

Test Description

- Triplet repeat-primed polymerase chain reaction (PCR) followed by size analysis using capillary electrophoresis to determine *FMR1* CGG repeat length
  - Methylation-specific PCR analysis is performed for CGG repeat lengths of 55 or greater
    - Methylation analysis is used to distinguish between premutation and full mutation alleles
- For detailed descriptions of methodological considerations for fragile X testing, refer to ACMG Standards and Guidelines for Fragile X Testing (Monaghan, 2013)

Tests to Consider

Primary Tests

Fragile X (*FMR1*) with Reflex to Methylation Analysis 2009033

- Preferred test to diagnose fragile X syndrome and carrier screening in individuals with a positive family history

Fragile X (*FMR1*) with Reflex to Methylation Analysis, Fetal 2009034

- Prenatal test for fetuses of mothers with fragile X premutations or full mutations
- Fetal testing for normal or intermediate maternal alleles is not recommended

Related Test

Genetic Carrier Screen (CF, FXS, and SMA) with Reflex to Methylation 3000258

- Screen for genetic variants that indicate carrier status for cystic fibrosis (CF), fragile X syndrome (FXS), and spinal muscular atrophy (SMA) in pregnant couples or those planning a pregnancy
- Do not use for diagnostic testing in patients with symptoms of CF, FXS, or SMA

Disease Overview

Prevalence

- Disease prevalence
  - 1/4,000 males
  - 1/8,000 females
- Premutation allele prevalence in U.S.
  - 1/1,000 males
  - 1/350 females

Symptoms

- Neurologic
  - Moderate, mild, or asymptomatic in females
  - Moderate to severe intellectual disability in males
- Behavioral
  - Hyperactivity
  - Perseverative speech
  - Social anxiety
  - Poor eye contact
  - Hand flapping
  - Autism spectrum disorder
- Characteristic appearance of adult males
  - Macroorchidism
  - Long, narrow face
  - Prominent ears and jaws
  - Single palmar crease
- Connective tissue anomalies
  - Hyperextensible finger and thumb joints
  - Hand calluses
  - Velvet-like skin
  - Flat feet
  - Mitral valve prolapse
- Fragile X-associated tremor/ataxia syndrome (FXTAS)
  - Older premutation males (less commonly premutation females)
  - Progressive cerebellar ataxia
  - Intention tremor
- Premutation females may develop POI
- Early diagnosis with early intervention likely maximizes outcomes

Genetics

Gene – *FMR1*

Inheritance – X-linked

Structure/function

Produces RNA-binding protein, fragile X mental retardation protein (FMRP)

Expressed in many tissues

Mutations

- Trinucleotide CGG repeat expansion is cause of FMRP deficiency in most individuals
- Risk for repeat expansion is dependent on sex of transmitting parent and size of allele transmitted
  - Full mutation – >200-230 CGG repeats (methylated)
    - Males are affected
    - 1/3 of females are typically affected, 1/3 are mildly affected, and 1/3 are unaffected
    - Not possible to predict disease severity based on
      - Size of CGG repeat
      - Degree of methylation
      - Pattern of X-inactivation (in females)
  - Premutation – 55-200 CGG repeats (unmethylated)
    - Transmission unstable in females
    - May expand to full mutations in offspring
    - Premutations of >56 repeats have not been reported to expand to full mutations in a single generation
  - Intermediate – 45-54 CGG repeats
    - Unstably transmitted – more likely in families of affected individuals than general population
  - Normal – 5-44 CGG repeats
    - Stably transmitted
- Mosaicism may reduce disease severity

Test Interpretation

Sensitivity/specificity

- Clinical sensitivity/specificity – 99% (GeneReviews)
- Analytical sensitivity/specificity – 99% (Lyon, 2010; Grasso, 2014)

Results

- Full mutation (>200-230 CGG repeats [methylated]) – FXS
  - Mental retardation in males
  - Variable expression in females
- Premutation (55-200 CGG repeats [unmethylated])
  - Male – at risk for FXTAS and will transmit premutation to all daughters
  - Female – at risk for POI, FXTAS, and having offspring with full mutations due to allele expansion
    - Nearly 100% of maternal CGG repeats of >90 expand to full mutations in offspring
- Intermediate (45-54 CGG repeats) – offspring at increased risk for being a premutation carrier
- Negative (5-44 CGG repeats) – allele size in normal range and methylation pattern consistent with individual's sex

Limitations

- Estimated size is not provided for full mutations with >200 repeats
- Methylation patterns are not fully established at the time of chorionic villus sampling for fetal testing
  - Amniocyte analysis is recommended to distinguish a small, full mutation from a large premutation
- Rare mutations in *FMR1* unrelated to trinucleotide expansion will not be detected
- Diagnostic errors can occur due to rare sequence variations

References

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Spinal Muscular Atrophy

Indications for Ordering

- Diagnostic testing to confirm a suspected diagnosis of spinal muscular atrophy (SMA)
- Prenatal or preconception carrier screening for SMA in the general population
- Carrier screening for reproductive partner of known SMA carrier
- Carrier screening for parents of a child with a deletion of the *SMN1* gene or other family history of SMA
- Quantify *SMN2* copy number for treatment purposes

Test Description

Multiplex ligation-dependent probe amplification (MLPA) to determine copy number for the *SMN1* and *SMN2* genes

Tests to Consider

Primary tests

Spinal Muscular Atrophy (SMA) Copy Number Analysis 2013436

- Diagnostic or carrier testing for SMA
  - Although relevant only for affected individuals, *SMN2* copy number will be reported for all patients
    - Chromosomal phase cannot be determined
  - Test also includes 2 *SMN1* variants that are part of a haplotype associated with *SMN1* duplication in silent carriers, especially in Ashkenazi Jewish and Asian populations
    - c.*3+80T>G (rs143838139), g.27134T>G
    - c.*211_*212del (rs200800214), g.27706-27707delAT
    - Relevant only in the context of carrier screening; however, will be reported for all patients as present or not present

Spinal Muscular Atrophy (SMA) Copy Number Analysis, Fetal 2013444

- Prenatal diagnostic testing for SMA when both parents carry a deletion of *SMN1* or have a previous child with SMA caused by a deletion of *SMN1*
  - *SMN2* copy number will be reported

Related Test

Genetic Carrier Screen (CF, FXS, and SMA) with Reflex to Methylation 3000258

- Reproductive carrier screening for cystic fibrosis (CF), fragile X syndrome (FXS), and spinal muscular atrophy (SMA)
- Recommended for women who are pregnant or planning a pregnancy
- Not recommended for men, as FXS carrier screening is not indicated
- Do not use for diagnostic testing in patients with symptoms of CF, FXS, or SMA

Disease Overview

Incidence – ~1/10,000 live births in the U.S.

- Carrier rate ~1/50 overall in the U.S.; varies by ethnicity
- See table for ethnicity-specific residual carrier risk

Symptoms

- Progressive muscle weakness due to degeneration of lower motor neurons
  - Clinical findings of affected individuals fall on a spectrum
  - Most common symptoms
    - Difficulty breathing, swallowing, and walking
- SMA subtypes are distinguished by age of onset and severity for purposes of prognosis and management
  - SMA 0 – prenatal onset
    - Most severe form, survival is typically <6 months
  - SMA 1 – onset at 0-6 months
    - Most common subtype of SMA
    - Severe muscle weakness, survival is typically <2 years
  - SMA 2 – onset at 6-12 months
    - Child usually cannot walk without assistance
  - SMA 3 – onset after 12 months
    - Milder muscle weakness, child usually can walk and stand without assistance
  - SMA 4 – adult onset
    - Mild muscle weakness, normal life span

Diagnostic testing

- Diagnosis is based on clinical findings and molecular genetic testing
  - Electromyography (EMG), nerve conduction velocities (NCV), and muscle/nerve histology may aid in diagnosis
- 95-98% of individuals with SMA have a homozygous loss of *SMN1* (zero copies of *SMN1*)
- 2-5% of individuals with SMA have loss of *SMN1* on one chromosome and a pathogenic sequence variant in the remaining copy of *SMN1* (not detected by this test)
- Not possible to definitively predict clinical subtype based on genotype
  - Higher *SMN2* copy number may correlate with milder disease severity in affected individuals

Carrier testing

- Presence of two or more copies of *SMN1* usually indicates patient is not a carrier, although residual carrier risk exists
  - Test is unable to determine if *SMN1* copies are on the same or opposite chromosome
- 3-4% of general population has both copies of *SMN1* on the same chromosome (also known as *SMN1* duplication)
  - If paired with *SMN1* loss (zero copies) on the opposite chromosome, these individuals are "silent carriers" or "2+0 carriers"
- Two or more copies of *SMN1* on the same chromosome is rare but more frequent in certain populations such as African American and Ashkenazi Jewish
- Two variants which are part of a haplotype associated with *SMN1* duplication are tested
  - Presence of two *SMN1* copies plus the variants suggests increased risk that the individual is a silent carrier but is not definitive
  - Silent carrier likely if patient is Ashkenazi Jewish or Asian American

Pathophysiology

- SMA is caused by low levels of survival motor neuron (SMN) protein essential for motor neurons
- Majority of full-length SMN protein production comes from *SMN1* gene
- Some full-length SMN protein production comes from *SMN2 gene*
  - *SMN2* differs very little from *SMN1*
    - Does not contain exon 7 which alters mRNA splicing and protein production
  - Usually multiple copies of *SMN2* per chromosome
    - Increased full-length SMN protein production from *SMN2* may partially compensate for SMN protein production missing due to altered *SMN1* gene
- Spinraza (nusinersen) is an FDA-approved drug that can be used to treat SMA by increasing the amount of full-length SMA protein produced from *SMN2*

Genetics

Genes – *SMN1*, *SMN2*

Inheritance – autosomal recessive

- 98% of SMA is caused by abnormality in both copies of *SMN1* gene

De novo rate – 2%, usually paternal in origin

Variants tested/reported

- *SMN1* copy number
- Variants tested for haplotype associated with *SMN1* duplication
  - c.*3+80T>G (rs143838139)
  - c.*211_*212del (rs200800214)
- *SMN2* copy number will be reported for all individuals

Test Interpretation

Sensitivity/specificity

- Clinical sensitivity for diagnostic testing (Hendrickson, 2009; Luo, 2014; Prior, 2013)
  - 95-98% of individuals with SMA have 0 copies of *SMN1*
  - 2-5% of individuals with SMA have 1 copy of *SMN1* plus a pathogenic sequence variant
- Detection rate for carrier screening
- See table for ethnicity-specific residual carrier risk

Results

- Diagnostic test results
  - Zero copies of *SMN1* detected
    - Consistent with diagnosis of SMA
  - One copy of *SMN1* detected
    - May have SMA if an undetected pathogenic sequence variant is present
    - At least a carrier for SMA
  - Two copies of *SMN1* detected
    - Not affected with SMA
  - *SMN2* copy number will be reported but cannot be used to predict the severity of SMA with certainty
- Carrier screening results
  - One copy of *SMN1* detected
    - Individual is a carrier for SMA
  - Two or more copies of *SMN1* detected
    - Carrier risk is reduced but not eliminated
  - Two copies of *SMN1* detected and positive for targeted variant(s)
    - Normal copy number but increased risk to be a silent carrier (may have both *SMN1* copies on same chromosome, no copies on the other chromosome)
      - Not definitive
  - See table for ethnicity-specific residual carrier risk

Residual SMA Carrier Risk Posttest Based on Ethnicity and Test Result							
Ethnicity	**Carrier Frequency**	**Detection rate for carrier screening**	**Interpretation based on this result: 1 copy of SMN1**	**Posttest (residual) carrier risk based on this result: 2 copies SMN1, linked variants present**	**Posttest (residual) carrier risk based on this result: 2 copies SMN1, linked variants absent**	**Posttest (residual) carrier risk based on this result: 3 copies SMN1**	**Posttest (residual) carrier risk based on this result: 4+ copies SMN1**
Ashkenazi Jewish	1 in 41	94%	Carrier	Likely Carrier	1 in 580	1 in 4,000	Low, unquantified
Asian American	1 in 53	93%	Carrier	Likely Carrier	1 in 702	1 in 5,000	Low, unquantified
African American	1 in 66	71%	Carrier	1 in 34	1 in 396	1 in 3,000	Low, unquantified
Hispanic American	1 in 117	91%	Carrier	1 in 140	1 in 1762	1 in 11,000	Low, unquantified
Caucasian	1 in 35	95%	Carrier	1 in 29	1 in 769	1 in 3,500	Low, unquantified
Sources: Hendrickson, 2009; Luo, 2014							

Limitations

- Diagnostic errors can occur due to rare sequence variations
- Single base pair substitutions, small deletions/duplications, regulatory region mutations, and deep intronic mutations will not be detected
- Test is unable to determine
  - Whether *SMN1* copies are on the same or opposite chromosomes
    - ≥1 copy of *SMN1* on each chromosome (not a carrier) indistinguishable from ≥2 copies of *SMN1* on 1 chromosome and 0 copies on the opposite chromosome (silent carrier)
  - Whether *SMN1* and *SMN2* copies are on the same or opposite chromosomes

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