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
    - One or more copies of *SMN1* on each chromosome (not a carrier) indistinguishable from two or more copies of *SMN1* on one chromosome and zero copies on the opposite chromosome (silent carrier)
  - Whether *SMN1* and *SMN2* copies are on the same or opposite chromosomes

References

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