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 SMN2 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

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Carrier Frequency</th>
<th>Detection rate for carrier screening</th>
<th>Interpretation based on this result:</th>
<th>Posttest (residual) carrier risk based on this result:</th>
<th>Posttest (residual) carrier risk based on this result:</th>
<th>Posttest (residual) carrier risk based on this result:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 copy of SMN1</td>
<td>2 copies SMN1, linked variants present</td>
<td>2 copies SMN1, linked variants absent</td>
<td>3 copies SMN1</td>
</tr>
<tr>
<td>Ashkenazi Jewish</td>
<td>1 in 41</td>
<td>94%</td>
<td>Carrier</td>
<td>Likely Carrier</td>
<td>1 in 580</td>
<td>1 in 4,000</td>
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<tr>
<td>Asian American</td>
<td>1 in 53</td>
<td>93%</td>
<td>Carrier</td>
<td>Likely Carrier</td>
<td>1 in 702</td>
<td>1 in 5,000</td>
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<tr>
<td>African American</td>
<td>1 in 66</td>
<td>71%</td>
<td>Carrier</td>
<td>1 in 34</td>
<td>1 in 396</td>
<td>1 in 3,000</td>
</tr>
<tr>
<td>Hispanic American</td>
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<td>91%</td>
<td>Carrier</td>
<td>1 in 140</td>
<td>1 in 1762</td>
<td>1 in 11,000</td>
</tr>
<tr>
<td>Caucasian</td>
<td>1 in 35</td>
<td>95%</td>
<td>Carrier</td>
<td>1 in 29</td>
<td>1 in 769</td>
<td>1 in 3,500</td>
</tr>
</tbody>
</table>

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